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GENETIC PERSPECTIVES IN DISEASE RESISTANCE AND SUSCEPTIBILITY

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GENETICS OF DISEASE RESISTANCE: INTRODUCTORY REMARKS

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It is a distinct honor to introduce a publication dealing with a subject that in my opinion has much greater long-time significance than either space exploration or increased knowledge of the interior of the atom. The most important thing in the world is the quality of life, especially of human life. To attain improved quality of human life, one of the most important tools placed in our hands is the science of genetics as derived during the past century from Charles Darwin and Gregor Mendel. Genetics may not be used to improve the quality of human life in your lifetime or my lifetime, but sooner or later sound conclusions will be drawn from the careful work done in breeding for disease resistance in plants and animals.

My own experience and observations have been in agriculture, in which I have had the opportunity of watching for generation after generation the changes in genetic resistance to disease in several different organisms. In all plant-breeding work the factor of disease resistance is usually a *sine qua non*.

The only practical approach to the red stele disease in strawberries is either growing the strawberries on disease-free land or growing resistant varieties. The same thing applies to *Verticillium* wilt, which affects both strawberries and tomatoes. Such diseases are harbored in the soil many years and are bound to become more important as the years go by unless combatted by genetic means. Fortunately there is a wealth of genetic material to combat such diseases.

In the case of gladioli two of the most important diseases are fusarium and botrytis. Here again we know the source or resistant material and are slowly incorporating it into material for the commercial grower.

In the case of sweet corn, the corn ear worm can be protected against by spraying with DDT every other day during silking time. However, it now appears that a certain strain of corn has in its silks a chemical substance unpleasant to ear worms. Therefore, this trouble may eventually be fought by genetic rather than by chemical means.

I think of the way in which a South Dakota farm boy made wheat resistant to stem rust. He crossed wheat of 21 chromosomes with Yaroslav Emmer of 14 chromosomes, a cross so violent that for several generations there was high sterility and most of the kernels were shriveled. It was only after seven generations that he developed a variety called Hope, which combined stem-rust resistance with high milling quality. Today the blood of this variety is found in nearly all the wheat grown in the great Northwest.

In oats there are many strains of rust and blight. The impact of the diseases changes constantly, and the oat varieties have to be changed as fast. For a time an oat from Russia was all the rage. Then a new type of rust and smut knocked it out, and Kherson was combined with an Uruguayan sort. After the new varieties had dominated the field for only a few years, there came a virulent attack of *Helminthosporium* or blight. To meet the new threat, blood from Algerian oats had to be incorporated. This process will go on and on as long as diseases change and there still remain untapped sources of genetic wealth. Time after time it may be necessary to go to wide species crosses with material that looks worthless but that has one or two priceless qualities.

In corn we find enormous differences in resistance to a wide variety of bacterial and fungous diseases. The various seed corn companies spend more than $100,000 each year breeding for disease resistance.

Some years ago the melon business was almost knocked out by mildew. Melon breeders searched the world for resistant sorts, and a gene bank was established. After some years of hard work, mildew resistance was successfully combined with commercial quality. The industry was saved only to be confronted with destruction by a virus. A new gene bank was hastily assembled, and again the new virus-resistant character was combined with commercial acceptability and agricultural productivity.

In citing these specific instances, the fundamental point I am making is the fact that nature and primitive man have developed in many parts of the world types of plants that may look unpromising in the commercial world of the west but that may have certain priceless qualities of disease or insect resistance. In corn we have recognized this fact by setting up rather elaborate gene banks in order to be prepared with adequate genetic wealth to meet a wide range of contingencies.

Animal breeders have been much slower than plant breeders in recognizing the possibility of breeding for disease resistance. Poultry breeders will probably lead the way. In our work at Farvue Farm, we find that some crosses year after year will have much less death loss from enlarged liver than other crosses. Whether because of sex linkage or cytoplasmic inheritance, it makes a difference which way the cross is made. There is some indication that Leghorns may stand both blue comb and hepatitis better than the heavier breeds. Many thousands of chickens have been blood-typed, and much work has been done to discover the blood types and combination of blood types that will produce the least mortality. The leads are extremely promising, but the results are not yet conclusive. We do know, however, that homozygosis at one locus almost invariably leads to the disappearance of the homozygous birds within a few generations. As a rule heterozygosis at most loci seems to pay off in chickens.

Logically gene banks should be established for chickens just as for corn and melons. So it goes for other animals. It might be that the Mongolian pony might have as much to contribute to the race horse as Yaroslav Emmer to wheat.

We know much about genetic diseases in both domestic animals and human beings. So much is known that it is essential that every veterinarian and every doctor should have a course in the types of hereditary weakness, whether they are simple Mendelian dominants or Mendelian recessives, whether they are sex-linked or autosomal. We know about the Rh factor, thalassemia, sickle-cell anemia, hemophilia, and color blindness. Queen Victoria, who was a hemophelia carrier, had daughters who married into the royal houses of Spain and Russia, and trouble resulted. It was because the Czarevich was a bleeder that Rasputin got his foothold. Without Rasputin there might have been no Russian Revolution.

However, in this monograph we are more interested in the inheritance of disease resistance than in the inheritance of genetic diseases. Here we get into areas where the inheritance is usually complex and outside the realm of simple Mendelian analysis. Sooner or later many relationships between blood types and susceptibility to certain diseases will be discovered. They will be complex even though the blood types themselves are inherited in a simple Mendelian manner. The statistical presentations that have appeared thus far are stimulating but not conclusive. Blood typing was started on its way about 60 years ago by Karl Landsteiner and his students at about the same time that Mendelism was rediscovered. It took 20 years to relate blood types with Mendelism. Another 20 years went by before it was discovered that about 1 of 200 babies was dying because the father had one blood type called Rh positive and the mother had another called Rh negative. In another 15 years it was found that women of O blood type married to men of B or A blood type had fewer surviving children than the reciprocal mating. These broad statistical generalizations do not apply in specific instances.

For instance my second son and myself are both B blood type, and both of us are married to O blood type wives. There are a total of 7 healthy children and no miscarriages. On the other hand, my sister with O blood type is married to an A blood type husband and was bothered by miscarriages until she had two children. In other words, I am not at all certain that we should urge O type women to avoid B and A type mates or Rh negative women to avoid Rh positive mates. I am certain, however, that the study of blood types in animals and human beings will open extraordinary doors to knowledge. In the case of animals, I believe blood typing will more and more be a guide to successful breeding operations.

Growing along with our increased knowledge of blood types as they relate to disease will be our knowledge of the most important substance in the world, DNA.

Eventually both fields of knowledge will merge, and we shall then be on the verge of conferring on humanity "built-in" disease resistance. God grant that when and if this power comes to pass, we may use it more wisely than we have thus far used our knowledge of the atom.

This potentiality is probably several hundred years in the future. Perhaps the first suggestion of the eventuality came when it was discovered that the substitution of only one pair of valine molecules for glutamic acid molecules among several hundred resulted in sickle-cell disease. There are now suggestions from the Lafayette Clinic at Detroit, Mich., that a particular protein molecule in the blood may account for schizophrenia. It will take much more time before the chemistry and possible heredity are traced as completely as with the sickle-cell disease.

We are only on the fringes of real knowledge about the nature of both animal and human genetics as related to disease resistance. Nevertheless I am certain that we can eventually improve the quality of life by genetic means.

Today humanity is not wise enough to lay the foundation for its own genetic survival. I believe it is one of the most important activities in the world aside from seeing that we do not blow each other up or poison each other out of existence.

I am not at all certain that those human stocks most resistant to disease are superior in other respects. We still know almost nothing about the inheritance of genius. There is no likelihood that any nation or group of people will soon attempt scientific breeding of superior human beings on any large or continued scale. What to breed for, aside from general vigor, health, and mental awareness, we do not know. Sooner or later public records will be kept of those people who have hemophilia, sickle-cell disease, certain types of insanity, and similar hereditary weakness in their ancestry. Young people have the right to know what the odds are that a particular marriage will result in the possibility of abnormal children. This type of precaution is negative, not positive. We shall not be ready for positive steps until we have accumulated vastly more knowledge than we have today. Nevertheless, as a student of plant breeding and human populations, I know that time is running out on the human race unless research such as that presented in this monograph is taken very seriously.

In the case of plants the excessive use of monoculture and fertilizers has greatly increased the need of breeding for disease resistance. Usually genes have had to be picked up from several different localities in order to serve the purpose of both health and commercial acceptability.

The modern environment of human beings has changed more in the past 3 generations than in the previous 50 generations. Tens of millions of human beings are being saved now who in the time of our grandparents would have died without leaving children. Public health and antibiotics have created a new environment that will slowly but surely change the genetic character of humanity. The environmental changes are very rapid, but the genetic response is slow. I should not worry too much if I could be sure that public health, antibiotics, and other medical measures could continue to improve. What I fear is that we shall develop a civilization that will greatly increase our load of bad genes while at the same time public health and medicine becomes static or regresses. Man as a genetic organism would then find himself sufficiently out of balance with his changed surroundings so that irreversible disaster would be imminent.

Today we do not know enough to direct the course of human evolution upward. In another generation education about human genetics may have reached enough people so that positive action may be taken to arrest the downward trend in our inheritance. The quality of human life everywhere on this globe is a precious thing. Our objective everywhere is to improve the environment, maintain liberty, and gradually to take steps to reduce the increasingly heavy genetic load occasioned by the propagation of those with serious mutational disorders. This monograph is a faint precursor of the increased knowledge that must be more and more a part of our national consciousness.

In conclusion, may I say that the spiritual, artistic, and emotional qualities in man will long seem far more important than disease resistance. There is even a bare possibility that disease susceptibility will conduce to these high qualities. Our greatest human beings have not always been perfect physical specimens. We must never forget the divine spark that characterizes man at his highest, if not always his healthiest.

Part I. Adaptation in Man and Animals

THE "HOST FACTOR" IN DISEASE: GENETIC AND ENVIRONMENTAL INTERACTION

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The medical literature is replete with references to the so-called "host factor,"* giving notice of a general recognition that "it takes more than a microbe to produce a disease," and that "other causes are to be found in the characteristics of individual members of the population attacked, as well as in the environments in which both host and parasites, 'or other causative agents,' find themselves" (Paul, 1959[1]). Aside from drawing attention to these salient facts and entering a plea for the early development of satisfactory methods of analysis, until quite recently there was little to be said concerning the host factor that was of pertinence to epidemiology. By bringing experimental genetics into the focus of epidemiology it is hoped that this monograph will stimulate the development of methods suitable for the analysis of differences in host resistance and susceptibility.

Today there are unquestionably few who have not been indoctrinated with at least some of the basic principles of genetics and who are not fully familiar with the genetic terminology as it may apply to their particular area of interest. In this day of specialization, however, a problem arises: for within different areas of interest even basic terms and concepts take on specialized meanings that, while serving the purposes of a particular area of interest, may lead to confusion and misconception if applied in other, although intimately related, branches of the same discipline. This is exemplified by use of the terms genetic and nongenetic disease so frequently employed in biochemical genetics.

To the uninitiated the terms genetic and nongenetic disease imply a contradiction of the concept of genetic-environment interaction and has the unfortunate effect of resurrecting from its long overdue grave the notion that disease is inherited. No characteristic is inherited, normal or abnormal. It is only the genetic material that is inherited, and it is this that sets the individual's norm of reaction: the limits as to how and to what degree he will react to any or all of his environmental life experiences, physical and psychological. These experiences include both the adequacies and inadequacies of the individual's nutrition, exposure to infection and contagion, the condition of his prenatal life, accidents in development, and even his age.

Far from being an academic quibble, distinction between what is inherited, character or gene, is essential to our understanding of the host factor in by far the majority of disease states. At any given time, the host, the individual, is the summation of his life experiences as realized within the limits of response set by the total of his genetic endowment. The range of this response extends from the two possible extremes. At the one extreme are the single gene alterations (mutations) that impart their effects in all genetic backgrounds and in all environments (these are the situations to which the biochemical geneticist may as a shorthand convenience apply the term genetic disease). At the other extreme the effect of the genotype may be detectable only by statistical methods and then only within a limited environmental constellation. Between these extremes fall many well-known examples; a familiar one is that of erythroblastosis fetalis: an Rh-negative mother, if previously immunized by other Rh-positive pregnancies or by an Rh-positive blood transfusion, may develop antibodies to the red blood cells of an Rh-positive fetus. These antibodies may then return to attack the red blood cells of the Rh-positive fetus and result in hemolytic disease. Required is a single major gene difference, but one that will produce disease in the proper uterine environment only under a specific set of circumstances and when preceded by the prerequisite environment. These are not the only well-defined events that are required, for only 1 in 20 Rh-negative women carrying an Rh-positive fetus becomes sensitized.[2]

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* The term host factor is used here in accordance with the popular meaning in Preventive Medicine; disease is taken to mean any impairment of health.

Despite the shorthand designations that geneticists use in referring to genes and equating gene action with specific genetic traits, many (and probably most) traits are affected by more than one gene,[3] particularly at the level of biological organization at which the majority of common disease states are defined. At this level of biological organization it is also of particular importance that the action of hereditary factors be evaluated within the context of the environments in which they are expressed.

Because of the difficulties encountered in studying man in his varied and complex environment and the relative ease with which "unit characters" can be handled, much of our knowledge concerning the genetics of human disease is confined to those relatively rare entities that are fully conditioned by single major gene differences in the majority of "normal" environments. Xeroderma pigmentosum provides a good example: there is a simple Mendelian segregation of a major gene; homozygotes for this gene exposed to ultraviolet rays of the sun suffer severe deterioration of the skin and, possibly, death at an early age. This disease is the result of the interaction between the expression of a major gene and a normal and readily defined environmental agent. Such single gene differences, however, are not representative of the bulk of the genetically conditioned variability observed in either health or disease. Most variables of common observation are genetically complex[3-8] (for example, eye color) and are in addition subject to modification by a variety of environmental influences, as in the case of stature.[3-8] It is in situations of this complex nature that difficulties arise and the generalized term "host factor" may be used to denote individual differences in disease susceptibility. The host factor, as this term is commonly employed, is not synonymous with "genetic factor," for included are other features—phenotypic features such as nutritional status and body temperature. These characteristics are the result of genetic-environment interaction; consequently, the concept of a host factor does not distinguish between determinant and effect or genotype and phenotype. This is essentially a descriptive term and, for the purposes of objective analysis, it is necessary to distinguish between genetic and environmental components of the variability described. By the application of genetic methods of analysis this is possible, and considerable progress has been made in a number of instances where both a definable genetic factor and a causative agent or specific environmental situation are prerequisite to the disease process. Some examples of these in man and other animals are presented in the papers following. In many other instances, of course, it has not yet been possible to partition the host factors in such a way as to provide satisfactory explanations for observed differences in individual susceptibility to disease, for example, in many forms of cancer or cardiovascular disease, both of which are important subjects of epidemiologic investigations.

When a disease, as it is clinically described, fails to show clear-cut Mendelian ratios within affected families or to have simple causative explanations that always result in a comparable impairment of health in all individuals subjected to its influences, the usual procedure is to single out the most likely diagnostic criterion for genetic study. A good example is that of blood pressure, which is of proved value in the description of disease and has been frequently made the object of genetic investigation. Clinical evaluation of the measured level of the blood pressure is based upon population norms and the total of the circumstances in which a particular reading or set of readings are obtained. In the early studies of this variable, attempts were made to take clinically defined ranges of normal and abnormal readings as discrete traits. Studies by Pickering,[9] Miall and Oldham,[10] and others have since shown that blood pressure is a continuously distributed variable and that whereas the measured level of the blood pressure may have a genetic component of variability, environmental influences such as nutritional status and stress are also of great importance.

In a recent collaborative study Osborne *et al.** employed the twin-study method in the analysis of blood pressure. Twins provide a particularly sensitive technique for partitioning the hereditary and environmental components of variability of such attributes. In that study of disease-free adult twin subjects, basal blood pressure gave a very poor indication of a genetic component of variability. The same was true when casual blood pressures were employed. Further interpretation of these data led to the conclusion that there are genetic influences that affect blood pressure readings but that blood pressure measurements per se poorly describe the genetically conditioned variables. (A full report of this study has been prepared for presentation elsewhere. I cite it here only to provide an example of the character abstraction that may be represented by such critical variables as the measured level of the blood pressure.)

The fact that medically useful and reproducible measurements can be conveniently obtained does not provide assurance that the critical expression of either a genetic or environmentally influenced process has been described. Such measurements are only the outward signs of a complex array of physiological functions and responses; they are not discrete genetic or physiological characters; they are only abstractions used to facilitate the description of certian observations.[3] Blood pressure is a reading taken with the aid of a mechanical device and represents an end point of a long and complicated chain of biological processes in relation to an untold number of environmental influences. To understand the genetic and environmental forces reflected in measured levels of the blood pressure it will be necessary to define more effectively the processes this measurement purports to describe.

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* Osborne, R. H., F. V. De George, and J. A. L. Mathers, "The Variability of Blood Pressure: Basal and Casual Measurements in Adult Twins" (in preparation).

A second and somewhat different situation is exemplified by the measured levels of the serum cholesterol. The serum cholesterol is of interest because of its probable relationship to atherosclerotic cardiovascular disease. We are all acquainted with the reports in the press and in the professional literature of the differences in prevalence of atherosclerotic disease in different areas and among different peoples: atherosclerotic changes being relatively more prevalent in advanced societies, particularly among professional and higher income groups (for example, physicians in New York City) as compared to groups with a marginal subsistence (for example, Bantu of South Africa). Parallel differences have been observed in serum cholesterol levels, which have been in turn related to differences in fat intake.

Family studies have indicated that both atherosclerotic disease and excessive serum lipid levels are familial. Gedda and Poggi[11] very recently reported twin-study evidence on the genetic regulation of the serum cholesterol. These investigators studied 50 monozygotic and 50 dizygotic twin pairs ranging from 6 to 19 years of age. As comparisons were made on intrapair differences, environmental factors were controlled since both members of each twin pair were living in the same home with their parents at the time of study. The statistical analysis was based upon classifications of concordant and discordant pairs. Remarkable agreement was obtained for the monozygotic twin pairs: 37 pairs agreed perfectly in their cholesterol levels, whereas only 12 pairs of the dizygotic twins showed absolute agreement. This difference in concordance between monozygotic and dizygotic twin pairs is highly significant. According to Gedda and Poggi this gives evidence of "the genotypic dependence for the biogenesis of the cholesterol."

In an earlier study of 43 monozygotic and 39 dizygotic adult twin pairs, Osborne *et al.*[12] obtained results that differed rather interestingly from those found by Gedda and Poggi. These adult twin pairs ranged from 18 to 55 years of age. For the purposes of the analysis twin pairs were subdivided according to sex and zygosity into two groups representing twin pairs living together and living apart prior to and at the time of study. Twin pairs were classified as living together if the two members were sharing the same home and taking at least two meals a day together.

Monozygotic twins living together were compared to dizygotic twins living together, and monozygotic twins living apart were compared to dizygotic twins living apart. The dizygotic differences were found to be consistently larger than those of the monozygotic twins, but in no instance was the difference statistically significant; they were certainly far short of the magnitude of differences reported by Gedda and Poggi.

How might we account for the contrasting results from these two studies? Possibly greater differences in the external environment between the two members of an adult twin pair, even when living together, overshadow the genetic control of serum lipid[13] levels to be observed in children: for example, differences in occupational stress,[13] which are not equated in a common domicile. Or, possibly, there is also something more fundamental than this. Serum cholesterol levels are sex- and age-dependent; children between the ages of 6 and 19 years are undergoing rapid growth and development; and adults are undergoing not only the effects of more complex environment but also have an adult physiology. Illustrated here are: (1) the importance of the environmental factor of age with all its potential ramifications; and (2) measurements of even a relatively well-defined phenotypic character such as serum cholesterol do not necessarily represent an expression of the same biological processes when obtained under different environmental conditions.

The complexity of the interrelationship of genetic and environmental forces illustrated by these examples is extremely important, but within the usual context of medical investigations I am most concerned with another fundamental aspect of this problem that is also illustrated by these data. The intrapair variances for total cholesterol, cholesterol esters, and phospholipids of the twin pairs living apart are consistently larger than for those living together in all zygosity and sex categories. When the data are arranged according to both the genetic and environmental similarity of the twin pairs, the cumulative effects of these two forces are clearly seen. In other words, monozygotic twins living together have the most similar serum lipid levels, and dizygotic twins living apart the most dissimilar; between these two extremes are to be found the monozygotic twins living apart and the dizygotic twins living together. It is apparent that variations in serum lipids result from an interplay of genetic and environmental influences; it cannot be presumed that genetic factors will be equally important in all environments, or that the environmental tolerances of one population will necessarily equal those of another population.

To recognize the role of the host in the etiology of disease the nature of genetic-environment interaction must be understood. In all probability this applies to the majority of diseases and to the attributes by which a disease can be defined: attributes that reflect deviations in the "normal" processes by which man has achieved adaptation to his varied environment.

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PROBLEMS IN THE ENVIRONMENTAL ADAPTATION OF DOMESTIC ANIMALS

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The disease state is a specific disturbance arising in or superimposed upon an animal organism already profoundly involved in maintaining an equilibrium of function in the face of varied, variable, and often conflicting demands placed upon it by its environment. To meet these demands, complicated or not by a disease state, the animal has only those potentialities permitted to it by its genetic inheritance, developed or modified in the course of its past individual history.

The purpose of this paper is to examine: (1) the nature of the animal's struggle in the face of environments that threaten its thermal equilibrium as well as other aspects of its well-being; (2) to indicate what might be done to improve the situation for both the animal and its proprietor; and (3) to suggest (only by raising the question) the role that genetics might play in improving the economic returns to be obtained from domestic animals under adverse environments.

The Energetic System

The domestic animal is essentially a factory or chemical plant for the conversion of the potential energy of feed into forms desired by the proprietor, such as milk, meat, eggs, fur, or work. As with all factories it is economically desirable that the ratio of useful output to raw material input be as high as possible. The fact that passage through an animal body is inherently a less efficient way of getting food energy from the soil to man than by direct consumption of crops simply makes questions of animal efficiency more acute.

The diagram in FIGURE 1 sets out the broad nature of the energy flow. The potential energy of feed (A) is converted by metabolic processes (B) into the potential energy (C) of products (including the animal's growth), external work (D), or heat (E). The problem is to direct the flow into those channels that give the best returns to the proprietor, having regard to the inherent nature and limitations of the animal mechanisms and also to the special constraints imposed upon the system by the particular circumstances of operation: that is, to the genetic and environmental parameters.

Animal adaptation. I shall not here attempt even a simplified description of the extremely complicated metabolic processes by which the potential energy of feed is converted into its final forms. Suffice it to say that the process takes the form of an oxidation, with a certain amount of conversion to heat as an inescapable phenomenon, and that the process is regulated at every turn by enzyme systems that are more or less direct progeny of the genes operating in the various cells of the animal body. The final partition of energy between work products and heat, although often in response to recognizable environmental stimuli, is mediated through the same complex system of enzymatic, and therefore gene-derived, controls.

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The Thermal Balance

Heat is an inescapable and often a major end product of the energy conversions; and, since temperature exercises an important effect upon the relative rates and net result of cellular processes, it is important that investigators be familiar with the mechanisms by which the rate of heat production and the rate of heat loss from the animal and its tissues are kept reasonably balanced.

Heat production. The rate of heat production can be broken down into segments according to convention or convenience. For the purposes of this paper the following divisions are useful: basal component, habitual component, daily maintenance component, performance component, and management component.

The basal component or, more accurately, the rate of heat production measured under standard resting conditions, contributes from 35 to 70 per cent of the average daily heat production, depending upon the magnitude of the remaining components. The rate for most adult mammals is in the neighborhood of 40 kcal. /sq. m./hour. A relatively low basal rate should favor heat adaptation, but it must be remembered that the physiological conditions responsible for or accompanying a low basal rate may so reduce the yield that any expected economic advantage is significantly offset.

$$\left.\begin{array}{l}\text{Potential}\\ \quad\text{energy of}\\ \quad\text{feed (A)}\end{array}\right\}\longrightarrow\left\{\begin{array}{l}\text{Metabolic}\\ \quad\text{processes}\\ \quad\text{(B)}\end{array}\right.\left\{\begin{array}{l}\text{Basal state}\\ \text{Habits}\\ \text{Daily maintenance}\\ \text{Performance}\\ \text{Growth and reproduction}\\ \text{Management}\end{array}\right\}\longrightarrow\left\{\begin{array}{l}\text{Energy of external}\\ \quad\text{work (D)}\\ \text{P.E. of products (C)}\\ \text{Heat (E)} \rightarrow \text{Loss to en-}\\ \quad\text{vironment (F)}\end{array}\right\}$$

FIGURE 1.

In the habitual component I include the additional heat production due to the habit patterns of the animal, such as its tendency to stand quietly, run about, or take fright. The importance of "temperament" is easily observed in hot-room studies, where the body temperatures of excitable individuals may soar whereas those of their more placid companions remain within acceptable limits.

The daily maintenance component is the additional heat production resulting from the very complex set of chemical changes that follow the taking of food: alimentary movements, glandular secretion, absorption from the gut, transformations and storage of absorbed nutrients, and increased cellular activity following the addition of metabolizable material. The term specific dynamic action is used somewhat loosely to cover some or all of these processes.

The performance component is the additional heat production brought about by the processes that give man what he seeks from the animal: that is, milk, eggs, meat, fur, or work. Included in this category are the necessary prerequisite functions of reproduction and growth.

By the term management component I wish to draw attention to the additional heat production that may result from management practices. In this category falls the additional heat production caused by long distances of walking for food or water, feeding of fibrous or other foods that induce a relatively high heat production in proportion to the nutrients obtained, moving over rough terrain, as well as exposure to biting insects and other irritating environmental conditions. All of these components of total heat production are subject to some control and should be considered in optimizing the thermal situation.

Heat loss. The most obvious mechanism used by animals for adjusting heat loss to the total thermal situation under hot conditions is variation in respiratory activity. An increase in the volume of air respired gives increased opportunity for the evaporation of water from the respiratory tract, and thus greater heat loss; however, there are certain drawbacks. In the first place, breathing involves muscular action and heat production, which offsets to a certain extent the cooling produced by evaporation. A more serious difficulty, however, is that excessive breathing may wash carbon dioxide out of the blood passing through the lungs and induce an "alkalosis" with disturbances to general cellular function. This risk may be minimized if the rate of respiration is increased while the depth of each respiration is decreased. Then there are mechanical and physiological limits beyond which the respiratory effort cannot be pushed. Actual fatigue of respiratory activity can be demonstrated in hot-room studies.

A less noticeable method of promoting heat loss under hot conditions is by evaporation of water from the skin. Few animals other than the horse produce obvious sweat in the way that man does, but measurements show that large quantities of water are actually evaporated from the skin of many mammals. There is good reason to believe that the sweat glands present in the skin of animals, although different in many respects from those of man, do in fact participate in this process.

By comparison, all other methods of controlling heat loss under hot conditions appear to be relatively minor. The thinning of the coat in summer is of some advantage but only if the air temperature is below that of the blood. Appendages facilitate heat loss only if they have a large and superficial blood flow. Rabbit ears and rat tails are probably effective, but chicken combs and bovine dewlaps are apparently not.

Some animals are not without ingenuity in making up by behavior what nature has failed to provide by automatic mechanisms. Most animals will seek shade when they need it. Most will use water, if it is available, either for bulk cooling or for evaporative cooling. Cats, Australian "possums," and some others will lick themselves for the cooling effect, but the pig may stand and stare at a growing pool of saliva without being moved to action. Recumbency reduces heat production but obstructs heat loss. Some animals seem to know when it is advantageous, but in others lassitude may pass into coma without any apparent break in behavior.

Heat regulation. The mechanism by which heat loss and heat production are kept in virtual balance is substantially the same in all birds and mammals, although the means at their disposal vary somewhat in relative usefulness, and the range of thermal environments in which reasonable balance can be maintained varies widely.

The paramount control is located in the hypothalamic area of the forebrain in a series of nerve-cell clusters referred to collectively as the heat-regulating center. These cells obtain information on the thermal state through sensory nerves from the skin and through the temperature of the blood passing through the center (there is still some uncertainty about the relative importance or conditions of importance of these two sources of information). The cells of the heat-regulating center exercise control over the heat balance in various ways: by nervous stimulation of the respiratory center, sweat glands, and skin blood vessels; by collateral effects upon brain "centers" controlling posture, activity, and sleep; by nervous control of tissue metabolism through the sympathico-adrenal system; by nervous effects upon the pituitary functions and thus upon a wide range of endocrine and tissue activities. The extent to which these various mechanisms operate almost certainly varies with the circumstances and probably also with the species. Much remains to be learned about their detailed modes of operation.

Problems Imposed by the Physical Environment

I now refer again to the diagram of energy flow (FIGURE 1) to see what stresses various types of physical environment impose upon the system and to determine exactly the problems that face the animal in those environments.

Warm, humid environments. By reason of the relatively high temperatures, but more particularly by reason of the high humidities, a restraint is imposed upon heat loss to the environment (F). The best solution to the primary problem, therefore, would be to have animals with the ability to increase heat loss until full compensation is obtained. To the extent that this solution is beyond the power of the animal, then one needs animals whose heat production (E) can be reduced, but without adversely affecting the output of useful products (C) or work (D). Inasmuch as the feed available to the animal in warm, humid environments tends to be high in fiber and imposes a relatively large heat production upon the animal in obtaining the desired nutrients from it, a high efficiency in the rest of the metabolic processes is a particularly desirable feature.

The energetic qualities desired in animals for warm, humid environments therefore may be characterized as: (1) good mechanisms for increasing heat loss; (2) high efficiency in conversion of food energy to useful products; (3) ability to reduce heat production without reducing the production of useful products; or (4) ability to tolerate rise of body temperature without loss of functional efficiency.

Hot, dry environments. The high temperatures interfere with heat loss (F), but the low humidities may offset this in animals with good evaporative mechanisms. To the extent that these mechanisms are not adequate, the animal is in much the same situation as one in a warm humid climate, in that reduction of heat production (E) is desired without reduction of useful product (C, D). To the extent that evaporative mechanisms are effective, the animal may be faced with the secondary problem of water conservation. To the extent that food is scarce, as well as fibrous, efficiency in extracting nutrients is more than ever desirable.

The list of desirable characteristics for animals under hot, dry conditions thus become fairly formidable: (1) insulation against solar energy and hot winds; (2) good evaporative mechanisms, but with other means of conserving water; (3) very high efficiency of converting food to useful products; (4) ability when necessary of reducing heat production without reducing useful products; or (5) ability to tolerate rise of body temperature without loss of functional efficiency.

Cold environments. The thermal problem in cold environments is the reverse of that in hot environments. Because of low temperatures and possible wetness or wind, the rate of heat loss (F) is often higher than the unprovoked resting metabolic rate of the animal. It is clearly desirable that this rate of heat loss be checked, preferably by high insulation through a thick or dense coat. To the extent that this optimum is not met, a higher rate of heat production (E) is required; this, however, threatens to increase the food requirement (A) and thus to reduce the economic value of the animal. This threat may be circumvented, however, if the heat production is secondary to useful performance (C, D). This would be true in high producing animals, thus increasing the importance, always dear to the proprietor's heart, of good productive individuals. (The efficiency of conversion, which was a prime requirement for animals in hot conditions, is rather less important here than the total metabolic turnover.) A high art of foraging and self-preservation may be needed in snow-covered or fog-bound areas.

Thus the desirable characteristics for cold-weather animals are: (1) high coat insulation; (2) minimum of exposed extremities that have either to receive a thermally wasteful blood supply or to run the risk of frostbite; (3) high productive rate; (4) good foraging capabilities; and (5) what herders call "range sense."

High altitudes. It is only in the South American mountains that temperatures and forage permit large numbers of animals to graze at altitudes (over 7000 feet) where the lowering of barometric pressure really becomes a problem. Hand-fed animals, however, may be kept at relatively high altitudes in the northern hemisphere. The problems here are those of cold conditions, complicated for range animals by a precarious fodder supply and a relative shortage of oxygen. The oxygen deficiency, if uncorrected, leads to reduced or faulty metabolic conversion (B) of food, with lowered yield of useful products (C, D), and perhaps with lowered heat production (E) under conditions where a high rate may be essential. Secondary consequences may be leakage of fluid from anoxic capillaries into the pendulous tissues (brisket disease) and impoverished heart function, both making matters worse for the animal. Reproductive functions are often depressed under those conditions.

In addition to the requirements for cold-weather animals, those to be maintained at high altitudes need: (1) increased oxygen-carrying power in the blood through increased concentration of red blood cells or an altered hemoglobin dissociation curve; (2) ability to transfer oxygen from the capillary blood to tissue cells at a lower partial pressure; (3) a high efficiency in extracting nutrients from food; and (4) absence of any condition that will interfere with any step in the complex transfer of atmospheric oxygen to the active cellular metabolic processes. The autochthonous animals of the Andes seem to have some of these characters genetically established, whereas animals of lowland origin show no more than phenotypic adaptations to varying degrees in different species.

Present Methods for Meeting the Problems

Now that the main problems of maintaining animals under adverse environments have been reviewed, the question is: What can be done about solving these problems? Three approaches may be attempted: (1) improvement of the performance of existing stock; (2) immediate acquisition of better stock; and (3) breeding of better stock. To simplify the presentation these will be examined in relation to hot environments, but the principles are largely applicable to all adverse environments.

Improvement of existing stock. To the extent that one is concerned with high economic return in existing animals, one needs to concentrate on those factors of environment or management that tend to increase the heat burden or to reduce the animal's resistance. Shade in the pasture, air movement and ventilation in barns, easy access to water and feed, reduction of foods that stimulate undesired heat production, relegation of peak performance as far as possible to cooler seasons, and use of simple cooling devices have all been advocated at length in the scientific and technical literature.

Acquisition of better stock. This presumes that better stock can be recognized. Historically, this presumption seems to be traversing three interesting and somewhat chastening phases. In the first it was presumed that certain breeds or types could be found that had a definitely superior "heat tolerance" to others. Experience seemed to justify this presumption. Zebu strains of beef cattle seemed to be more heat tolerant than European breeds; White Leghorn hens appeared more resistant than Australorps; and camels are much more tolerant of desert conditions than horses.

There is a fairly voluminous literature on the heat tolerance of different species, breeds, and types of domestic animals. A few years ago the Food and Agriculture Organization of the United Nations attempted to reduce this information to a meaningful comparative statement, but almost insuperable difficulties arose. In the first place, a large proportion of the data was not accompanied by information that enabled one to evaluate the role of adventitious circumstances such as the nutritional plane, level of production, extent of prior acclimatization, or conditions of management. Unfortunately, where such information was given, there was no knowledge with which to reduce these variables to a common denominator. Even some of the attempts to standardize conditions of measurement failed to take into account important conditions such as the rate of air movement or the intensity of sunshine.

Beyond such technical inadequacies of available information, however, a serious philosophical doubt arose. Where the most careful and extensive studies had been made, it became clear that significant differences in heat tolerance could be established within a species between certain groups bearing breed or type names. It was by no means clear, however, that the groups studied were typical of the animals classified under those breed or type names. Merino sheep studied in Australia behaved differently from Merino sheep studied in the United States; Jerseys in Louisiana did not correspond at all points with Jerseys in Maryland. It seemed a little bold to make assertions about Red Sindhi cattle in general from intensive studies on a handful collected at the United States Agriculture Research Station at Beltsville, Md.

Moreover, intensive studies showed that there was a wide range of variation in heat tolerance within the members of a supposedly uniform group even when measured under carefully controlled and standardized conditions. The mean tolerance of Sindhi-Jersey F_1 crosses is significantly greater than that of pure Jerseys, but there are some Jerseys with a higher tolerance than some Sindhi-Jerseys. As long as the concern is with the average behavior of well-defined breeds or types, information as to average tolerance is presumably useful; but if a foundation stock is being set up, it is the qualities of the individuals that assume importance.

Furthermore, the concept of breed or type is a predominantly Western or technological one. If there is a wide range of variation in physiological behavior within a supposedly uniform Western breed or type, how much more variation should be expected in the heterogeneous flocks and herds of regions unaccustomed to "pure" breeding? To rely on supposed averages of supposed types is optimism twice removed.

Thus a study that was begun to define the comparative tolerance of groups concluded by convincing its sponsors of the importance of the individual.

More attention has since been given to what I should now call the *second historical phase*: the search for the more tolerant individual; yet this too seems to have difficulties and dangers of its own. Exactly what is meant by "heat tolerance," especially in the individual?

To the animal producer the definition of heat tolerance is fairly simple; it is the ability of the animal to maintain its useful performance under hot conditions. To the physiologist the problem does not appear as simple, for he is aware of the numerous nonthermal as well as thermal factors that may influence performance; he also wants to know more precisely the basic mechanisms involved and the way in which various factors may affect them. Five types of tolerance mechanism may be envisaged: (1) increased heat loss; (2) decreased heat production; (3) resistance to the effects of rising body temperature; (4) reduced secondary consequences of heat-regulatory processes; and (5) resistance to the secondary consequences of the heat regulatory processes. The superior heat tolerance of one animal over another, in the producer's sense, may have its basis in one or all of these mechanisms.

The producer has the simpler concept of heat tolerance, but he has some difficulty in measuring it since the yield must be observed over a fairly long period of time to avoid the adventitious effects of transient circumstances. It frequently happens that by the time the heat tolerance is determined in this way, the problem for that particular animal has resolved itself. This type of information is most useful to the extent that it reveals the probable genetic tendencies for tolerance that the individual *may* pass to its progeny. To this point I shall return later.

The physiologist hopes to discover some recognizable characteristic in the animal that is so closely associated with the determination of tolerance that it will serve as an index that will lend itself to early and quick measurement before much time and effort have been invested in the animal's food and maintenance. The most useful characteristics thus far studied are rise of respiratory rate and rise of rectal temperature. These can be observed quite easily in the laboratory and fairly easily in the barn or field. Other reactions, of perhaps greater physiological interest, such as rate of water evaporation from the skin or respiratory volume, do not lend themselves to field measurement and require fairly complex equipment in the laboratory. Of the really fundamental characteristic—efficiency of conversion of food energy to useful production—little is known and simple methods of measurement are lacking.

Now I refer to the *third phase*, which is the newest, the most challenging, and perhaps eventually the most revealing: the search for the key energetic processes that ultimately determine both the performance and the thermal well-being of the animal. Attention must be focused upon the whole range of energy-exchange processes, from the consumption of food, through the biochemical processes of utilization, to the caloric significance of growth, products, and excreta. From a detailed and intimate knowledge of these transformations the key processes may well emerge, and with the knowledge, the ability not only to recognize the most suitable animal, but also to regulate and perhaps order these processes at will. Inasmuch as they are enzymatically controlled and gene-determined, genetics, cytology, enzymology, thermodynamics, and physiology will unite in the service of the animal producer.

Breeding better stock. To be successful in breeding it is necessary to be able to recognize the distribution of the desired genetic characteristics in the potential foundation animals and to be acutely aware of the laws under which those characteristics are transmitted and interact to favor the appearance of the desired qualitities in the offspring.

It is clear from what has been said that the desired characters are not easily defined or selected, even phenotypically, and little is known of their genetic transmission. In the absence of more precise knowledge it is assumed that "tolerance" is a multifactor trait that tends to be distributed with other multifactor characters in statistical fashion; thus the hope for really fresh and occasionally useful combinations is by segregation. To the extent that one is prepared to rely on these two nebulous principles—recognition of desirable results and the occasional pleasant surprise from segregation—the following guides may be suggested:

(1) Selection among indigenous stock capitalizes on segregation that has occurred naturally. This is applicable where good types occur. Improvement may be rapid at first but diminishes with successive generations.

(2) Grading-up, by crossing high-producing but less tolerant imported animals on to tolerant but low-producing local types, is suitable for large numbers. It is a long-term process. Segregation is likely to occur when progeny are interbred.

(3) Maintenance of fixed ratio in a cross between imported and local types imposes a breeding pattern that has to be continually repeated. Segregation is likely to occur when progeny with the desired ratio are interbred.

(4) Deliberate segregation is exemplified in the simplest form when the F_1 generation from high-producing but less tolerant imported, and tolerant but low-producing local types are interbred. From the resultant widespread variation in the F_2 generation the few highly desirable individuals may be used as foundation stock for a new line. This is a high-risk, high-payoff proposition, and very large animal populations must be used if the occasional dramatic success is to be exploited.

(5) Adaptation of imported types over successive generations is the only course open where local types are relatively worthless. The drop in production after importation will vary from slight to drastic.

Conclusion

Knowledge in this field is in a rather unsatisfactory state. Not much is yet known about the detailed mechanisms that control the conversion of the potential energy of food into either useful products or heat. While certain breeds or types may in general maintain better production under adverse conditions, the range of variation within the breed or type is large, and the results with individuals may be disappointing. Therefore, it is difficult to determine the most tolerant individual except by observing its production under one specific set of conditions, which gives only empirical knowledge and usually too late in the animal's life. It is assumed that tolerance is transmitted as a multifactor character; this in turn suggests that segregation is the only hope of a radical change, and then only occasionally is it in the desired direction.

If there is a way out of this impasse, it is through knowledge: knowledge of the genetic regulation of key processes in the bioenergetic complex and knowledge of what to look for in foundation stock and how to use it in a rational breeding program.

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BIOLOGICAL ADAPTATION OF MAN TO HIS ENVIRONMENT: HEAT, COLD, ALTITUDE, AND NUTRITION

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In meeting the challenge of the climatic extremes of heat and cold and the geographic extreme of high altitude man, like other animals, has the principal problem of maintaining his operating equilibrium. In nutritional terms there is a necessary metabolic balance to be accomplished. Beyond cultural paraphernalia and know-how, adjustments to maintain balance are accomplished by the bodily physiology, which is itself reflected in morphology. In very large part these physiological and morphological adjustments represent continuous variables of probably complex reactive origins. Consequently they are assumed to be under polygenic control. For this reason the evolutionary model, based as it is on the behavior of single alleles, is applicable only with extensive modifications to the human traits responding to heat, cold, altitude, and dietary stresses. In these traits, mutation and drift should have less discernible effect. Homozygosity and heterozygosity have different and lesser meanings, at least within the breeding patterns common in man. Selection and its product of adaptation, however, can operate rapidly in polygenic traits, particularly with the loading of linkage and recombination.

To a considerable extent, however, variation in these polygenic traits falls outside the evolutionary model because of the range of nongenic plasticity responses in man. It may be, as Hulse (1960) suggests, that the individual range of plasticity response is genetically based and that ranges differ within and between human populations. In any case the greatest problem in interpreting the physiological and morphological adjustments to environmental extremes is to decide whether they are plasticity responses or the result of selective processes. As an example: Are the special biological abilities of Eskimo to live in the high Arctic and of Nilotic peoples to cope with low-latitude savanna heat due to selective genetic processes, lifetime plasticity acclimatizations, or both?

This synthesis is primarily concerned with the bodily adjustments of native peoples to the environmental extremes; it is not designed to cover the voluminous literature on short-term acclimatization in whites. Since coordinated physiological and morphological studies are lacking for native peoples living in extreme environments, the two subjects are best dealt with separately.

Morphological Adjustments to Heat and Cold

Body size and proportions. The principle basic to Bergmann's size rule is that ". . . in otherwise similar bodies, the larger one has the smaller skin surface in proportion to mass, since volume and mass increase as the cube of the linear dimensions and surface only as the square" (Allee and Schmidt, 1951). Greater body size therefore increases the body-mass/body-surface ratio, and it is consequently of some advantage where the retention of metabolically generated heat is desirable. More laterally built bodies with shorter extremities further increase the mass/surface ratio in cold-stressed people like the Eskimos, in accordance with Allen's rule. On the other hand, the body-mass/body-surface ratio decreases with warmth of climate (Schreider, 1951) and often seems to be expressed by small body size in tropical forest peoples and by more linear body builds in low-latitude desert and savanna dwellers.

These regularities of body size with climatic temperatures have been demonstrated for the world by Roberts (1953), for the New World by M. T. Newman (1960) and even for the United States military population by R. W. Newman and Munro (1955). In the body-mass/body-surface ratio, Schreider (1953) showed a climatic gradient from 38 to 32 kg./sq. m. of skin in a large group of native samples. In calculating skin surface, Schreider used the DuBois height-weight formula, which because of body-build differences overestimates the skin surface of colder-climate peoples and underestimates it in warmer-climate ones (Newman and Jones, unpublished data*). Thus a more realistic human mean range in body proportions should be from about 30 to more than 40 kg./sq. m., with a known mean range for body mass from 39 to 74 kg. in male samples. Although climate is not the only regulatory factor for human body size and proportions, it seems to be the major one. In New World natives about 50 per cent of the mean variance in body mass is due to mean coldest-month temperatures. When mean statures are added to the mass-temperature relationship (thus affording a measure of body proportions) almost 80 per cent of the mean variance is accounted for (M. T. Newman, 1960). The residual variances, then, would seem due to other factors such as nutrition and health status also related to climate.

It is unfortunate that detailed body-build and composition studies have not been carried out on strongly heat- and cold-stressed populations. Hence we have no clear idea of the human range in these respects, and misinformation creeps in as well. Rodahl's (1952) study of skin surface area in a miscellaneous Alaskan Eskimo sample is a case in point. In this study average skin surfaces calculated by the height-weight formula were only 0.9 per cent higher than those calculated by the more accurate linear method. One possible conclusion is that these particular Eskimos are so similar in body build to whites that the height-weight formula is applicable to all Eskimos with minimum error. Nevertheless these particular Alaskan Eskimos are unusually linear in build for their people; other Eskimos are heavier and more laterally built. In both body mass and proportions, Eskimos show their own climatic gradient from the low to the high Arctic (M. T. Newman, 1960).

Subcutaneous fat. Baker (1959) states that if blood flow to the body surface is scanty, the total body is insulated according to the thickness and distribution of the subcutaneous fat layer. Such insulation would appear to be advantageous where retention of internally-generated heat is a major problem, just as it seems disadvantageous for heat dissipation. Accordingly, without much evidence other than common observation, it has been assumed that cold-climate peoples have thick layers of subcutaneous fat and that hot-climate peoples have thin layers. Empirical data from skinfold studies, however, suggest that over-fed and underworked whites carry as much as or more subcutaneous fat than any other people in the world. In Western peoples class is therefore the largest factor. With the age factor controlled, Spanish laborers have about one half the skinfold thickness of Spanish professional men (Keys *et al.*, 1954). For non-Western samples, North Australian aborigines, Sudanic Nilotes, Highland Peruvian Indians, and Formosan Chinese have about one half to two thirds the skinfold thicknesses of roughly comparable white series. With little question the Kikuyu in Kenya studied somatotypically by Danby (1956) had little subcutaneous fat, but nutrition must have been a heavy factor since the jail sample was appreciably heavier in weight than the "free" sample. Although Eskimos are alleged to be well supplied with an insulative subcutaneous covering, no skinfold or soft tissue X-ray studies have been done on them. Outside of facial fat padding, it is W. S. Laughlin's observation (personal communication, 1958) that Eskimos are not particularly fat.

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* Newman, M. T. and C. R. Jones, "The Biology of the Wai Wai Indians of British Guiana" (unpublished).

The best study on subcutaneous fat thickness and distribution was done on United States white and Negro soldiers by R. W. Newman (1956). Age for age the Negroes have less subcutaneous fat, and this fat has a different bodily distribution. Undoubtedly the contrast between white and Negro skinfold thickness would have been greater if Africans had been used; yet, as Baker (1959) has shown, even the same amount of subcutaneous fat in United States Negroes does not provide as efficient insulative protection as in whites. Cold-room studies showed the Negroes to have lower surface and core temperatures after exposure at 50° F. Further reference is made later in this paper to the cold-stress difficulties of Negroes.

Skin pigmentation. Man rather closely follows Gloger's pigmentation rule, based upon observations on other mammals and birds, which states that melanin increases in varieties living in warmer, moister climates and that dwellers in arid regions tend to be yellow or red-brown in pigmentation. World skin-color maps for man (Biasutti, 1953) show Gloger-type gradients in the natives of both hemispheres. Relatively unpigmented skin may have selective advantage in cool cloudy regions, but the only explanation thus far is that it permits ultraviolet penetration, facilitating production of irradiated ergosterol as an antirachitic agent (Coon *et al.*, 1950). In areas of extreme heat and light, however, heavily pigmented skin is a decided advantage. A. V. Hill (1954) noted in South Africa that white cattle suffer more from heat fatigue than black cattle. Thompson's (1951) work in Nigeria showing that sunburn in Europeans can be associated with real damage to the sweat glands; the high incidence of rodent ulcer in Queensland whites and the close association of malignant epithelial tumors with lighter-skinned people in Africa and South America (Blum, 1955) make it clear that melanin deposits are a valuable protection against ultraviolet radiation.

While highly pigmented skin absorbs more ultraviolet radiation that would be injurious to underlying tissues, it is also said to absorb more heat. This was once thought to be advantageous since the extra infrared absorption stimulated an early onset of sweating; yet, as Thompson (1955) says, since the relatively greater absorption would continue throughout subsequent exposure to solar radiation, this is a high price to pay for a dubious initial advantage. Following

Herr's (1952) calculations, relatively untanned Negro skin absorbs 34 per cent more solar energy than white skin. This extra heat load might constitute a significant strain on the thermoregulatory mechanisms, especially sweat production, under conditions of severe stress. The significance of such an overload has not been demonstrated, although on a matched-pair basis a small sample of United States Negroes was not superior to whites in preserving thermal equilibrium in the Yuma desert (Baker, 1955*a*).

It is notable that the experiments on reflective and absorptive qualities of the skin are referrable to unexposed, untanned skin and do not take into account the reactive hyperkeratosis of the stratum corneum under continued solar radiation. This reaction results in the formation of a thicker layer of more adherent and more keratinized horny lamellae. There is a distinct possibility that hyperkeratinized skin has enhanced reflectance qualities under any conditions. Moreover Salthouse's (1958) experiments with reflection and absorption of near-infrared radiation found that two very dark Nilotes actually reflected more from the skin surface than the one white control. Two dark Bantu showed the same surface reflectance as the white. The dark-skinned subjects, however, absorbed much more near-infrared in the stratum corneum and germinativum and permitted much less to reach the dermis than did the white subject.

Sweat glands. In man, as in other animals, the only means of dissipating body heat in an ambient temperature warmer than the body surface is by evaporation of water. Thus in areas of extreme heat, an efficient sweat physiology is a necessity for survival. On the other hand, it is very important for cold-climate people to deal properly with the problem of insensible respiration and, after a rate of energy production dependent upon ambient temperature is reached, with actual thermal sweating (Mitchell and Edman, 1951).

Incomplete data from Glaser (1934) suggest that in the majority of bodily regions, Bantus have more sweat glands than European whites. Thompson's (1954) work on Nigerians and whites failed to confirm the earlier researches. Instead he found tremendous individual variation in sweat-pore number for both races and no definite evidence of different distribution patterns. In East Asia, however, Kawahata and Sakamoto (1951) report a quite remarkable regression toward more sweat glands in peoples of warmer climates. Estimates in the 1000s are given in TABLE 1. Only 58 subjects were studied, and these showed high individual variability; the Ainu ranged from 1069 to 1991 thousands, but their low mean quite befits an originally boreal people. On the other hand, the difference between Japanese in Japan and those raised in the tropics strongly suggests that sweat-gland density is a plasticity response. The contrast between the lack of Negro-white differences in sweat-pore number studied by Thompson and the quite regular climatic gradient seen in Kawahata and Sakamoto's researches suggest difficulties of a technical nature.

Physiological Adjustments to Heat

The classic experiment on racial differences in heat-stressing is the Robinson *et al.* (1941) study of Negro and white sharecroppers in Mississippi. The Mississippi Negroes, who incidentally had a lower mass/surface ratio, showed a lower sweating rate than the whites; the former also maintained more normal core temperatures. Thompson (1954) also found lower sweating rates by 0.07 l./sq.m./hour in Nigerians versus European troops, and lower core temperatures as well under moderate stress conditions. Baker's (1958*a*) 40 pairs of United States Negro and white soldiers matched for stature, weight, and calculated body fat showed core temperatures significantly lower at the 0.01 level in the Negroes after an hour's walk at 3½ mph under fairly hot and humid conditions.

The many heat-stress studies make it clear, however, that valid interracial comparisons are possible only when the level of acclimatization to the immediate working conditions is carefully controlled and where large enough samples are studied to take care of high individual variation in heat response. In fully acclimatized Nigerians, Ladell (1951) noted initial sweating at lower rectal temperatures and higher sweat production per unit area but, after long exposure, a falling off apparently due to sweat-gland fatigue in a technical sense but representing a water and mineral conserving mechanism. Both in the laboratory and in the mines, however, the Nigerians were less susceptible than whites to heat cramps and prostration despite the low salt intake of the former. Controlled studies in other African mines showed the same fall-off of sweat production during long heat exposure in native workers (Weiner, J. S., 1950; Wyndham *et al.*, 1952), also attributed to sweat fatigue. No deleterious effects from this fall-off were noted, and it may represent an advantageous economy. Similar sweat-rate studies on small samples of Asians and acclimated British in Singapore showed somewhat lower rates in the former (Adam *et al.*, 1953). Wyndham and Morrison (1956) found that Bushmen hunters in the Kalahari and fully acclimatized Europeans were on a par in heat tolerance under only moderate stressing. Under more severe desert stress it may be that the Bushmen would show thermoregulatory superiority. These are the people who quite literally run the hoofs off antelope by keeping them on the move for days across scorching sand.

TABLE 1
COMPARISON OF HUMAN SWEAT-PORE ESTIMATES

Sample	Number*	Mean and S.E.
Ainu	12	1443 ± 52
Russians	6	1886 ± 59
Japanese in Manchuria	4	2186 ± 11
Japanese in Japan	11	2286 ± 66
Filipinos	10	2800 ± 23
Japanese born in tropics	15	2961 ± 61

* Numbers represent thousands.

No equatorial desert people have been studied for their abilities to withstand heat-stressing. With little doubt the heat tolerance of people such as the itinerant Solubbies of the Arabian desert is quite exceptional since, as Baker (1958*b*) points out, the external heat load forced on desert-dwellers is several times greater than in hot jungles. Baker's (1958*c*) "ideal" desert man—

linear in build, low in subcutaneous fat, and brunet in skin color with high tanning potential—describes the Solubbies exactly. Although no physiological data were collected, D. F. Roberts (personal communication, 1960) considers the Nilotes of the savannas of the Sudan to be the best adapted in the world to hot-dry heat. Failing a native desert sample, Baker (1958*a*) tested eight matched pairs of United States Negroes and whites in the Yuma desert where ground temperatures are as high as 150° F. Clothed subjects, sitting or walking, had about equal tolerance as regards race. When they were nude, suntanned whites had higher tolerance in terms of their lower core temperature. Thus American Negroes of predominantly West African extraction have superior tolerance to hot-humid climates but not to hot-dry climates.

The most definitive criterion of desert heat tolerance is survival. Directly bearing upon this criterion is Schickele's (1947) compilation of data on 157 deaths from heat prostration occurring during troop training and maneuvers in United States desert areas during World War II. Using height-weight figures from the fatal cases, she found that the greater the adiposity, the lower the heat tolerance. In addition high fat-free weight per unit stature—that is, lateral body builds—also reduced heat tolerance. Schickele was even able to make the following odds: Men 5 to 14 pounds over "normal" weight were 4 times more susceptible to fatal heat-stroke than men of "normal" weight; and men of average weight were 4 times more susceptible than men 16 or more pounds under the means for their age and stature. Regrettably no physiological data are available for these fatal cases, but the body morphology alone demonstrates an amazingly high selective value in unacclimatized subjects under high heat-loading.

Physiological Adjustments to Cold

It has been known for some time that the lowest air temperature at which naked men can maintain core temperature without metabolic increase is 25° to 27° C. (77-81° F.). At lower temperatures, core temperature is kept in equilibrium at metabolic cost. This metabolic acclimatization to cold may be a tropical-type emergency reaction as Scholander (1955) says, but a marked ability to generate large amounts of internal heat can have high selective value in the cold.

Since the most critical body areas in exposure to cold are the extremities, research emphasis has been on the deep and surface temperature of the hand or foot in ice baths. Brown and Page (1952) found that Southampton Island Eskimos maintained a higher blood flow to the hand, and consequently a higher surface temperature, than a control group of whites using ice baths regulated at 30°, 20°, 10°, and 5° C. Both groups showed initial vasoconstriction followed by vasodilation, but vasodilation occurred more strongly and at a higher skin temperature level in the Eskimos. At the same time the Eskimos showed a lower deep-hand temperature, a clue suggesting that some sort of nontropical insulative reaction may be present. This could be interpreted as an increase of partially cooled insulative shell, the most vulnerable surfaces kept warm enough to stave off frostbite.

Similar ice-bath experiments were repeated on Bering Sea Eskimos, Fort

Yukon Indians, and United States white and Negro troops by Meehan (1955). The Eskimos and Indians had the least cooling, the whites intermediate, and the Negroes the most cooling. Elsner *et al.* (1960) showed that Old Crow Reservation Indians had a markedly superior ability over urban whites to maintain warm hands in cold water. In Manchuria, Yoshimura and Iida's (1952) ice-bath researches yielded the following finger-temperature ranking (from warmest to coolest): (1) mountain-dwelling Tungus; (2) native-born Mongols and Chinese; and (3) resident Japanese. Furthermore, among both Chinese and Japanese there were subrankings by the temperatures of their places-of-birth.

In the ice-bath experiments, the white controls were either relatively unacclimatized such as Brown and Page's McGill medical students or United States troops in Alaska with only short-term acclimatization at best. The question as to how warm fully acclimated whites can keep their hands has only recently been answered by the work of Krog *et al.* (1960) on Norwegian Lapps and Norwegian fishermen. These two samples showed more rapid vasodilation than in the control group of physiologists, but at the end of 20 min. the blood flow was the same in all three groups. It is worth noting that the mean January temperature for the Lapp homeland is −15° C. (5° F.) and for the Norwegian fishermen −5° C. (23° F.), comparable only to the southernmost fringes of the American Arctic. Neither are a highly cold-stressed people, even compared with the low Arctic Southampton Island Eskimos whose mean January temperature is −24° F. Thus some discount may be taken from the suggestion made by Krog *et al.* (1960) that the higher blood flow rates found in the American Arctic peoples may reflect only a reduced vasoconstriction under low temperatures rather than anatomical differences in extremital blood supply. These differences, suggested in the arteriovenous anastomoses and the arteriovenae comitantes patterns (Lamb, unpublished data*), have yet to be properly investigated.

It is nevertheless true, as Daggs (1951) has said, that "the single most disadvantageous physiological property in a healthy individual exposed to a cold environment is early and intense peripheral vasoconstriction in the extremities, and a high resistance to release of this vasospasm once it is established." While this remark was not intended to describe the reaction of Negroes to cold stress, it sums up their position perfectly. The researches of Meehan (1955), Adams and Covino (1958), and Rennie and Adams (1957) show larger and more intense vasoconstriction in Negroes. Indeed, 6 of 8 of the United States Negro infantrymen tested by Rennie and Adams (1957) failed to show any vasodilation following the vasospasm in a 90-min. span in a 20° C. cold room. The absence of this normal "hunting phenomenon" or Lewis (1931) reaction in many Negroes would preclude the survival of a Negro population in the Arctic even under optimum Eskimo-style native living conditions. The experience of Negro troops in the cold is a case in point. Schuman (1951 to 1952) studied 716 cases of frostbite during the Korean War and showed that with all environmental conditions equal, Negroes were six times as prone to cold injury as whites and were also predisposed to more severe injuries. Meehan (1955) cites an Army exercise in interior Alaska in February, 1954, where 50 per cent of the cold injuries were in Negroes, who comprised only 10 per cent of the troops in the field.

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* Lamb, N. P., "Cold Stress and Race: Physiological and Anatomical Factors" (unpublished).

Further research on Negroes in the cold demonstrated that they had significantly higher cold agglutinin titers than whites (Weiner, 1952). The relationship of high titer agglutinins to frostbite is clearly one of intravascular clumping of red cells, slowing of blood flow, and occlusion of small vessels following exposure to the cold. Parenthetically, Weiner found that whites with histories of frequent upper respiratory infections had significantly higher cold agglutinin titers than those without such histories; also, that men with blood group O had the lowest titers, those with A or B intermediate, and those with AB the highest. If the blood testings are reliable (and surveys show as much as 15 per cent error in GI tags), these findings suggest that the presence of alpha or beta agglutinin affect the cold agglutinin titer about equally, but that, taken together, they affect it most.

Emphasis on keeping the hands warm in cold weather is not merely a matter of experimental convenience. No matter how warm their dwellings, native hunters of the Arctic must venture forth periodically for game. The degree of manual dexterity under such conditions probably represents the margin between procuring vital food and returning empty-handed. Coffee (1955) found that young Eskimos and Indian men were superior to acclimatized whites in ungloved dexterity under cold conditions. Le Blanc's (1956) study on impairment of manual dexterity in the cold emphasized the selective and survival aspects under critical cold-stressing.

The greater flow of warm blood to the extremities in cold-climate peoples presupposes some metabolic cost, although Carlson *et al.* (1953) suggest that an increase in insulative shell in acclimatized men reduces their metabolic needs. Wilber (1957), Milan,* and others feel that any metabolic differences pale into insignificance because of the artificial microclimate well-clothed and well-housed Arctic people carry with them at all times; yet there is a clear negative correlation of the order of $r = 0.5$ between basal metabolic rates and ambient temperatures in man (Roberts, 1953). In the Arctic various Eskimo samples show a mean range from +12 to +33 per cent of white norms, with a clear pattern of summer-time decreases (Bollerud *et al.*, 1950; Brown *et al.*, 1954). Rodahl (1952) claims that their high B.M.R.s are directly due to diet since 340 tests on 73 Eskimos subsisting on native foods showed a mean of +8 per cent; when they were shifted to white man's food, however, the mean dropped to −8 per cent. Beyond diet per se, the high B.M.R.s of Eskimos are due to their remarkable ability to metabolize the plentiful fats in their diet, which are enough to sicken most whites with ketosis.

The most remarkable metabolic adjustments thus far recorded, however, are for the Alacaluf Indians of Wellington Island at the tip of South America (Hammel, 1960). Only in the past 30 years have these Indians taken on white man's clothing. Prior to that they wore otter or seal skins draped over the shoulders.

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* Milan, F. A., "A Study of the Maintenance of Thermal Balance in the Eskimo" (unpublished).

In controlled "bag-test" experiments, 9 of these Indians showed resting metabolic rates elevated 150 to 200 per cent above DuBois adjusted standards and higher body temperatures than the white controls. This remarkable metabolic response to light-clad living in air temperatures around freezing permits these people to withstand even the chill of repeated diving after sea food in water from 6 to 8° C.

Mention has already been made of indications of an insulative cooling mechanism conserving body heat under certain thermal circumstances in Arctic people. Generally confirmatory are Elsner *et al.*'s (1960) findings of greater surface cooling of the arms and thighs but not the hands and feet of Yukon Territory Indians at the end of a cold winter. Nomadic Lapps also undergo a considerable drop in surface and core temperatures during sleep without the metabolic increase so typical of incompletely acclimated men (Andersen *et al.*, 1960). This is a heat-conserving reaction.

The most dramatic approach to true insulative cooling was found in Australian aborigines of the Western desert who sleep naked between small fires under the open sky with air temperatures about freezing (Scholander *et al.*, 1958). Under "bag-test" conditions simulating this cold-stress, the white controls were miserable, and they sleeplessly thrashed around all night; the aborigines, however, lay motionless and snored their way through the long hours. They accomplished this usually on a subbasal metabolic rate and with significantly greater cooling of the body surface—especially the extremities—than the uncomfortable controls. This insulative cooling under moderate cold is analogous to shutting down the heat and closing the door of an unused room to conserve fuel bills. It is likely that under greater cold-stress these aborigines would undergo metabolic adjustments that would sustain core temperatures at a tolerable level.

Morphological and Physiological Adjustments to High Altitude

High-altitude studies on native peoples are confined to the Andean area of South America, although the inhabited areas over 15,000 feet elevation in the Himalayas can provide a human laboratory of equal excellence. The problem of adjustment that man shares with other animals is that at 2 to 3 miles altitude the air contains only two thirds to one half the oxygen as at sea level, yet a proper supply to the bodily tissues must be maintained at all times. Otherwise the body develops an oxygen debt that, unless compensated, results in a circulatory-failure sickness that can lead to death (Monge, 1943). Beyond these pathological aspects there is a mounting literature on short-term acclimatization studies in which the United States Air Force is most active.

The best high-altitude studies on natives come from the laboratories of the Institute of Andean Biology in Peru (Monge, 1954). The natives are Quechua-speaking Indians, who may be the end product of as much as 10,000 years of high-altitude living. These particular Indians come from the Central Sierra of Peru and have rather recently acquired more whites' genes than those from other Andean areas. The easiest way to present the extraordinarily complex adaptative syndrome is in terms of averages for Indians living at 15,000 feet elevation. The facets to this syndrome are:

(1) Tremendous size of the chest, which seems to overshadow the rest of the physical development. Some Indians are even barrel-chested with a decided expansion of the lower part of the rib cage.

(2) Correspondingly, the lungs are large and are given even more space by a lowered diaphragm.

(3) Contrary to the sea-level state, the alveoli of the lungs are permanently dilated to afford greater surface for oxygen absorption; this expanded inner lining makes for a doubly-rich capillary bed capable of picking up more oxygen.

(4) There is a 40 per cent increase in blood volume due wholly to manufacture and maintenance of red cells (plasma volume is the same as at sea level).

(5) The red cells contain hemoglobin that absorbs oxygen via the alveolar capillaries. As with the expanded capillary bed, there is an actual doubling by weight of hemoglobin.

(6) Each red cell is somewhat larger than at sea level to afford maximum surface for oxygen absorption, but each enlarged cell contains somewhat less hemoglobin.

(7) Even so the arterial oxygen saturation is less (80 per cent) than at sea level (96 per cent). This means that at high altitude a lower proportion of oxyhemoglobin is in the arterial blood.

(8) There appears to be an increase in myohemoglobin (in general terms a companion substance to hemoglobin), which serves as storage for oxygen in muscle tissue and sustains a muscle from one contraction to the next. This concentration of myohemoglobin in high-altitude people is particularly notable in muscles of permanent activity such as the diaphragm and heart.

(9) The increase in red cells (plasma volume remaining the same) results in more viscous blood. As already mentioned a given amount of high-altitude arterial blood carries less oxygen. In consequence the heart has to pump more viscous blood and has less oxygen with which to pump it. At least partial compensation for these factors is provided by a larger heart, with a mean 20 per cent increase in transverse diameter in 400 subjects. This larger muscle, with clear signs of right-side predominance, is more competent to assume an overload.

(10) Even under moderate exercise, heartbeat is typically slow, and systolic blood pressures are also low. Diastolic pressures are normal, so the pulse pressure is decreased.

(11) It is conceivable that further relief for the heart is afforded by the typically compact build of Andean Indians, which should reduce blood-transport distances. Even so there are slightly delayed arm-tongue and arm-lung circulatory times.

As a consequence of hyperventilation, high-altitude people are capable of special respiratory adjustments that eliminate carbonic acid and maintain the fixity of the blood *p*H. In spite of increases in both acid and base in the blood, the *p*H is normal although slightly shifted to the alkaline side. The buffer capacity is increased so much that the *p*H is more stable at high altitude than at sea level for the same amount of work. In addition the increased blood flow would seem by casual observation to aid in keeping the extremities warm in a cold climate with a thinner atmospheric barrier to cold radiation through space.

Monge (1948) has written extensively about the "climatic aggression" of high altitude, which tends to keep the acclimated people in and the unacclimated ones out. The Spanish people as well as their domestic animals were infertile at first in the high country. The heaviest work in the mines is still done by Indians as a built-in labor force. Mestizos get along well at high altitude, but they have never been compared physiologically with the Indians. Thus there is no direct means of appraising how much of the intricate syndrome of adjustments to high altitude is due to selection and adaptation and how much to within-the-life-span plasticity.

One of the most striking demographic features of the high-altitude regions of the Andeans is the virtual absence of outsiders except on the managerial level. In large part, Asians and Negroes have not ventured into the more altitudinous areas of Colombia, Ecuador, Peru, and Bolivia. A hint that Negroes might expect extra difficulty at high altitude is provided by two studies in which either hemoglobin S or C, splenic infarction, and high-altitude flying were associated (Cooley *et al.*, 1954; Doenges *et al.*, 1954). In all only eight cases were investigated, and it is not clear whether one or both abnormal hemoglobins are the presumably responsible factor.

Bodily Adjustments to Nutrition

The geography-of-hunger philosophy held by some of the publicists in the nutritional field would have us believe that over one half of the world's population has a food intake barely sufficient to maintain life, yet in terms of fertility, work load, and longevity, this hypocaloric half of the world is doing a great deal better than hanging on to life. Most assuredly there are plenty of people in the world who are not getting enough to eat, particularly in terms of National Research Council standards. The most remarkable emerging aspect of nutrition, however, is that some human populations have made extraordinary adaptations to dietaries that would send many others to mass graves, even after due allowance for variables in body mass and ambient temperature. Gross morphological indications of such adaptations may be seen in the slow growth, late maturation, and resulting small body size of adults.

It is striking that the small-body-size end of Bergmann's rule and the hypocaloric part of the world coincide in the tropics. In many tropical areas crop yields are often poor because of thin, leached-out soils and negligent agricultural technologies. It is as though these low nutritional ceilings imposed upon tropical peoples add to the Bergmann effect in producing small people. Although less is known about nutritional requirements in the tropics than in any other broad zone, there are indications of extra stresses. Part of this argument concerns the dietary dilemma of putatively higher thiamine requirements in tropical man and lower thiamine content of tropic-raised meats as well as possibly higher excretory and other losses of iron and calcium in hot climates (Newman, M. T., 1953). Another part of the argument involves the concentration in the tropics of disease vectors that are parasitic in deriving sustenance from man's energy intake. For this reason as well as many others, many diseases are more severe in a synergistic sense in inadequately nourished people (Scrimshaw *et al.*, 1959). In consequence the tropics appear to constitute a broad zone of nutritional stress.

Other broad areas of nutritional stressing are not as conspicuous as the tropics but present equally great problems of public health. One such area is the highland plateau of Middle America, where there is still a heavy American Indian population. Adult body size is small in these people, and most samples are also linear in body build although there is fair evidence that in times past they were larger (Newman, M. T.*). Explanation for the small body size so currently characteristic may lie in the low per capita nutritional ceilings (Roberts and Newman†) and the hazardous disease environment consequent to the accelerative population increases without appreciable improvement in agricultural technology over the past several centuries. In Middle America, as in Highland Peru, growth is slower, maturation retarded, and adult body size small. This entire pattern may be viewed as an advantageous adjustment, perhaps due to both plasticity and selective responses to a quite hostile total environment. Experimental evidence in other animals shows that hypocaloric diets tend to inhibit production of the gonadotropic hormones and increase adrenal secretions (Boutwell *et al.*, 1948), suggesting a pituitary shift toward hormones necessary for survival at the expense of these essential for reproduction. The apparent result of this in man is a delayed maturation and pubertal growth spurt as well as a late attainment of small adult body size. Kralj-Čerček (1956) has even claimed that protein-rich foods accelerate maturation in man, while carbohydrates inhibit the process.

This brings to mind an axiom in the nutritional field that requires some emendation in the light of modern knowledge. According to this axiom, undernutrition (hypocaloric intake) rarely influences the basic health of a population, but malnutrition (avitaminosis and low mineral intake) can have grave clinical consequences. Unfortunately there is little information on the lower limits of tolerance of human populations, but there are some surprising instances of how well an adapted people can do under severe qualitative deficiencies. Nichols and Nimalasuriya (1939) report that although low-class Ceylonese have very low calcium intake there is no evidence of bony anomalies among them. The Quechua Indians of Vicos, Peru, have intakes of vitamin A and calcium of 4 and 14 per cent respectively of Instituto de Nutrición de Centro América y Panamá (INCAP) recommendations; yet with slow growth and maturation and low bone density, as measured by X-ray densitometry as an adjustment pattern, these Indians seem to do quite well (Schraer and Newman, 1958). Speaking of the Bantu, the Scientific Council for Africa South of the Sahara (1956) states: "The apparently low incidence of calcium deficiency disease, also of infantile scurvy, despite low intakes of the respective vitamins are challenges to the validity of the orthodox concepts in nutrition." One would think so; also that even with the usual compensations for sex, age, body size, ambient temperature, and work load, human dietary needs and tolerances vary widely the world over. The same may of course be said of the world's foodstuffs; a bean of given variety is not a bean the world over. For example, Ralph Brauer (personal communication) tested the food values of white potatoes in the market at Huancayo, Peru, and found as much as 300 per cent difference in these values.

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* Newman, M. T., "Evolutionary Changes in Body Size and Head Form in American Indians" (unpublished).

† Roberts, D. F. and M. T. Newman, "The Physiology of Middle American Indians" (unpublished).

If adjustment to food deprivation cannot be made, then death results. Where deaths due to food deprivation are culture- or class-limited there is of course differential survival, the *sine qua non* of biological evolution. A case in point is the high incidence of infantile and maternal beriberi in Upper Burmese of lower class, which resulted in much higher mortality than in Chinese and East Indians of the same communities (D. C. Sharma, personal communication). The vitamin B_1 deficiency was first imposed on the lactating Burmese mother by culturally-conditioned dietary restrictions and then applied also to the infant during the time for weaning. In Middle America, protein deficiencies have long been known to exist among many Indians and others of the bottom social stratum, but it took Scrimshaw and his co-workers (1957) to elucidate the whole problem. The protein deficiency (kwashiorkor) usually strikes near the end of the first year of infancy, when maternal milk may fail to supply enough protein, and supplementary foods — if given at all — are largely carbohydrates. The critical age, then, is from 1 to 4 years. By carefully checking the real cause of death in a large number of Guatemalan subjects, it appeared that for all deaths under 5 years of age, at least 25 per cent were due to kwashiorkor and, in the 1 to 4-year age group, at least one half the deaths seemed to be a direct consequence of malnutrition. Thus there would appear to be strong selection operating on the poorest and the most Indian, usually one and the same in Latin America.

As Neel (1958) has pointed out, nutritional overabundance can also be a killer. In our own culture, Neel says it is difficult to avoid the speculation that increasing levels of animal fat consumption as well as increase in total caloric intake, coupled with decreasing physical activity, have rendered heart disease an important agency of natural selection that formerly affected only a "favored" few. Dietarily the situation may be getting worse in view of Stanley Garn's recent description of United States child diets as more and more like a great big milkshake. Arteriosclerotic heart disease, including coronaries, account for over one quarter of the total United States mortality (Metropolitan Life, 1957), and it is hitting the young adults as well. Enos *et al.* (1953) reported that gross evidence of coronary atherosclerosis was discovered at autopsy in 77 per cent of young American soldiers (average age, 22 years) killed in action in Korea. If there is any genetic determinant in the ability to handle high dietary fat loads without developing atherosclerosis, then differential survival should be going on in our midst.

Conclusions

This cursory survey of human adaptation to environmental extremes necessarily involves phenotypic alterations of morphological and physiological traits that are largely continuous variables. Until their distribution curves can be solved, genetically speaking, the sort of polygenic blocks they represent must remain unknown. In as highly organized a form as man, however, these polygenic blocks may be composed of only a few genes of unequal effect rather than of many small genes of equal effect (Brues, 1950). Thus the means of perceiving the mode of inheritance of body size and proportions, skin pigmentation and sweat-gland function, heat production and the "hunting" phenomenon of vasodilation, oxygen pick-up and transport, and quantitative and qualitative dietary tolerances may be nearer at hand than we think. There are also a few hints of adaptive traits that may be controlled by single alleles. In the meantime, however, it may be best to heed Hulse's (1960) hypothesis that the more genes involved in the expression of a phenotypic character, the greater the chance that the character has a range of plasticity response. Thus most of the bodily alterations dealt with here could be partly due to within-the-life-span responses based upon a genetically-determined plasticity with different ranges for different human populations. The question as to whether selection operates directly upon the phenotypic traits themselves or upon their plasticity ranges cannot be settled here.

In any event the interpopulation variation of these phenotypic traits, in accordance with environment, is evidence of the *ex post facto* nature that evolution in this respect has gone on in man. Viewed in terms of the great blocks of humanity, it is apparent that Negroes do not do well in extreme cold and may not flourish at high altitude. They seem to be well equipped, however, to deal with heat, at least of the moist tropical forest variety. Mongoloid people, on the other hand, seem best equipped to cope with the cold; it may not be a matter of sheer coincidence that the high-altitude areas of the Himalayas and Andes are occupied by quasi-Mongoloid people. Mongoloids also thrive in the tropics of both hemispheres and as a whole seem to be the closest approach to an "all-purpose" man. Whites, who have pre-empted most of the best lands of temperate climate, do well in at least moderate cold and desert heat, but their history in the tropics leave them less than unqualified successes (Price, 1939).

In any case the adaptative range of the polytypic, polymorphic species of modern man is nothing short of tremendous. Part of this ability to get on anywhere and under the most varied conditions is due to man's unique possession of a culture. Whereas without clothing and with only little fires for warmth, man can withstand air temperatures below freezing, it is obvious that his entrance into the subzero areas of the Arctic had to wait until proper cultural paraphernalia was developed some 30,000 years ago. Extreme heat and altitude are endured only with special physiological and morphological adjustments. Culture does not help much. Human nutrition is in itself largely a cultural product except in the simplest human societies. Any human adaptation to the agricultural fruits of food production must have taken place only within the last 6000 to 8000 years. In short, adaptations of an essentially racial nature within our one species have had little operating time: a maximum of 50,000 years in geological perspective. Nevertheless, given sufficiently great environmental stressing, adaptation may be rapid. As a crude protective reaction to falciparum malaria, abnormal hemoglobin S may have taken less than 100 generations to reach a near equilibrium in its frequency (Livingstone, 1958). Polygenically controlled traits may evolve with equal rapidity under stress conditions.

It seems clear, at least in general outline, that the nature as well as the degree of human adaptations to those facets of the environment unrelated in a direct sense to disease may indeed reflect upon reactions to disease situations. There are many hints of this fuller relationship between man's adaptations to his climatic and nutritional environments and his reactions to disease that promise rich scientific dividends to the broad-front populational approach.

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ADAPTATION IN MAN AND ANIMALS: A SYNTHESIS

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Unless a synthesizer restricts himself to giving a rehash of the works he is supposed to synthesize, he must try to find a significant common denominator that is otherwise in danger of escaping the attention of the audience. The fine papers by Osborne, Lee, and Newman, however thought-provoking when taken separately, may seem to deal with scarcely related topics; yet they have conveyed in three different ways one basic message. I might attempt to restate this message as follows: certain classical dichotomies are no longer valid.

Dichotomies are persuasive, and they are dangerous because of their persuasiveness. We often speak and write that certain traits are hereditary and others environmental. The former come from the throws of the dice of destiny, and one cannot change one's heredity; the latter result from how people live and, at least in theory, people could live differently. It would greatly simplify matters if we could classify traits and determine which are hereditary and which environmental. This is impossible, because without exception all traits are hereditary and all are environmental. The color of skin is environmental, not only because one can tan himself or bleach himself by outdoor or indoor life, but also because to have any skin color one must have a skin, and the skin is a product of a process of development in which both genes and environment participate. Personality and social adjustment are influenced by heredity, not because a person is born with them and would have them regardless of how he lived but rather because other persons with other heredities, whose living experiences were approximately the same, developed different personalities and are better, or less well, adjusted to generally the same environment. How little these simple considerations are understood in some circles may be seen in an article published in a recent issue of a well-known periodical by two American physicians. In that article the authors argue that a certain disease cannot be hereditary because people sometimes recover from its attacks!

I do not mean to say that the distinction between hereditary and environmental diseases or between hereditary and environmental behavior traits is meaningless but, to be meaningful, the problem must be stated correctly. What we must ask is: What part of the observed variance in a given characteristic is due to the variety of genotypes existing in the individuals under study and what part to the variety of environments to which these individuals are exposed? This is the manner in which the question is asked by Osborne and De George[1] in their studies on the characteristics of twins. The answer obtained will be different for different populations and for different environments. The more uniform the environments the greater will be the observed heritability; the more genetically uniform a population under study, the greater will be the estimated environmental component. The apparent discrepancy between the results obtained by Osborne and by Italian investigators on the serum cholesterol levels is a case in point. The heritability of this trait may be different in different populations; the heritability observed may also depend on how one approaches the problem.

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Another spurious dichotomy is that between an adaptive and a neutral trait. People are all different from one another. One thing certain is that every person whom we meet is different from the one met before and from the one whom we shall meet next. Many of these differences are genetically conditioned (please note: I say genetically conditioned, not fixed by heredity!). The basic postulate of the modern theory of evolution, derived ultimately from Charles Darwin, is that organic diversity is one of the methods whereby life becomes adapted to the diversity of environments existing on our planet. Living beings are different because there are many different opportunities for life on this planet.

Does this mean that we must find an adaptive significance for every human trait? What is more useful: To have a nose of the shape I possess, or an aquiline nose, or a snub nose? A part of the difficulty comes from the misuse of the concept of "trait." A trait is an abstraction; the organism has no traits, it is our language that makes it necessary for us to describe living things in terms of "traits." The trouble begins when traits cease to be devices for description and analysis and become independently existing entities. This is what happened in the early years of genetics. Each gene was supposed to determine a single "unit-character" or trait of the organism. An early author wrote in 1910 as follows: "It has become evident that the individual is an entity made up of hereditary unit-characters. The aggregate of such units forms the individual. During the process of inheritance the units segregate, and by recombination they form new individuals."

The concept of unit-character is abandoned in modern genetics. There is no one-to-one correspondence between traits and genes. Many, and probably all, traits are determined by many genes; and many, probably all, genes determine many traits. A trait is an aspect of the total development pattern of the organism. It does not exist apart from this total development pattern.

Why, then, are people different? The matter seemed simple to pioneers of anthropology and biology. Genges L. L. de Buffon, Johann F. Blumenbach, and other 18th-century authors thought that people were modified by the environments in which they lived and that the modifications were inherited. A Negro was "a child of the African sun"; his skin got dark because he was suntanned for many generations. This deceptively simple theory has been accepted even to our day by many anthropologists, oblivious of the unfortunate fact that the theory assumes inheritance of acquired modifications and thus contravenes modern biology.

Race differences might have arisen also through natural selection as adaptations to the environments, physical and social, in which people lived. If so, the distinguishing characteristics of each race must be directly or indirectly helpful for survival and/or reproduction in the respective environments of these races. This at any rate is not obviously true; for example, what difference does it make whether your hair is straight, or wavy, or curly? Darwin hesitated to ascribe human race differences either to natural selection or to the

Lamarckian inheritance of acquired modifications; there is a third possibility: sexual selection. Gentlemen may prefer curly-haired blondes or straight-haired brunettes; ladies may have preferences for tall and slender or for smallish and pudgy gentlemen. If the tastes were unlike in different countries and if persons whose figures and features were popular with the opposite sex thereby produced more surviving progenies, then different races could have become different in appearance even though the race "stigmata" were not in themselves useful or harmful. Finally, the race differences may be due to genetic drift-accidents of sampling from the gene pool.

We are now in the unfortunate situation that evidence required to discriminate between the above possibilities is simply not available. To obtain it much painstaking work is doubtlessly needed. Newman is one of the pioneers in this field. The really startling fact is that this work has not been initiated until recently and, even now, not on a scale commensurate with its significance. This can be explained only by a lack of appreciation of the importance of the issue. To believers in Lamarckian inheritance, any form of selection, natural or sexual, is rather inconsequential. Some anthropologists went so far as to proclaim a principle that race classification must be based on traits that are neutral, that is, neither useful nor harmful to their possessors. The rationale behind this principle was that the classification should reflect the descent relationships of the races and that the characteristics induced by the environment, either through natural selection or Lamarckian-wise, are too labile to be dependable. The question of how neutral racial traits could have arisen and become established in the first place did not seem to have been asked or was not pressed.

Last but not least, the idea that human races differ in adaptively significant traits is emotionally repugnant to some people. An inquiry into this matter is felt to be dangerous, as it might vindicate race prejudice. This attitude almost invariably goes hand-in-hand with the misunderstanding of the nature of biological heredity repeatedly dealt with above. To be different is not tantamount to being superior or inferior. Furthermore, the differential adaptations of the races of man are most probably concerned with environments of a remote past, largely superseded by the environments created by civilization, to which all races may be equally adapted or equally unadapted.

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DISCUSSION: PART I

Frederick B. Hutt (*New York State College of Agriculture, Cornell University, Ithaca, N. Y.*): I have done work that bears on some of the subjects presented by Osborne, Lee, and Newman.

I am more hopeful than Lee about the prospects for breeding animals able to cope with environments that create stress in different parts of the world. As a geneticist, I am inclined to credit the good performance of the Santa Gertrudis less to the oil wells than to the astuteness and assiduity of the people at King Ranch, who experimented and eventually found a combination of three-eighths Zebu and five eighths Shorthorn, the merit of which has been attested not only in the southern United States but in Central America, Australia, and elsewhere, where these cattle have been tested at rather high temperatures.

The reason for my optimism is based partly in the fact that my prized White Leghorns are much more amenable to genetical investigation than Lee's prized camels.

In the fowl not only have we breed differences in capacity to tolerate high and cold temperatures, but we are able to modify these genetically as we wish. In Canada and the Union of Soviet Socialist Republics, fowls have been bred that radiate less heat by virtue of smaller combs than do chickens with large combs.

There are White Leghorns well able to tolerate high temperatures fatal to many heavy breeds such as Rhode Island Reds, Barred Rocks, and others. This fact has been confirmed not only in the United States but elsewhere; Lee found this to be true in Brisbane, Australia, long ago.

We are uncertain why White Leghorns are able to withstand high temperatures; nevertheless they do. Fox[1] shed some light on this. He found that if neither White Leghorns nor New Hampshires are given access to water, White Leghorns are no more resistant than the New Hampshires; but where White Leghorns were given water to drink, at high temperatures, they drank almost continuously and lived. The New Hampshires did not drink continuously and perished.

Although we may not have progressed in our knowledge of hereditary genius in man since Francis Galton wrote on that subject 80 years ago, these facts suggest that with chickens we might make progress in breeding for mental ability, enterprise, and ability to cope with their environment. Hens that know enough to continue drinking water possess a trait that may be equally as valuable as extra fat, extra radiation from the skin, or whatever it is that helps the camel. Of course, the hens cool off by evaporating water from the lungs and must continue drinking to accomplish this.

About 26 years ago, I attempted to learn why some chicks are able to withstand pullorum disease (caused by *Salmonella pullorum*) and others are not. It became evident that a genetically resistant breed (White Leghorn) is physiologically different in an interesting way from the susceptible breeds, the heavier breeds. The Leghorns have more critical control of their thermoregulatory mechanism. This is evidenced, among other ways, by a more rapid transition during the first 10 days after hatching from the poikilothermic state of the embryo, which is cold-blooded up to the time of pulmonary respiration, to the warm-blooded stage of a 10-day-old chick.

The birds resistant to pullorum disease react to infection by running a fever. Our test showed that the ones able to maintain this fever and withstand infection are the strains that can make most rapidly the transition from cold to warm blood after hatching.

The factors of environment and outer temperature are most important. Chicks inoculated with pullorum, and held at a brooder temperature of 28° C., die at the rate of 100 per cent, but if they are kept at a brooder temperature of 40° C., only 10 per cent may die.

Some years ago I undertook to breed chicks that could make this transition from the poikilothermic to the homoiothermic state more rapidly. The objective was to accomplish this selection without exposing the chicks to the disease, and then to test them for resistance. This has now been accomplished. By using good progeny testing, that is fast and efficient, two strains of White Leghorns were differentiated. In one of these the mean temperature, at 1 to 6 days after hatching in the second generation, averaged only 0.59° F. higher than that of the low temperature strain. Nevertheless, in 4 successive tests for resistance to pullorum, the high-temperature line was consistently and significantly more resistant to the disease than the low-temperature strain.

These genetical studies of coping with the environment lead me to believe that, with the right breeding methods and a little time, we shall breed domestic animals (I shall not extend this to man for various reasons) that can cope with their environment wherever they may be.

LEE: It is upon the significance of this problem for the future that I disagree with Hutt.

Reverting to the King Ranch, a real success was their spotting of the bull "Monkey," an achievement arising from 25 years of crossbreeding, during which time they were exploiting segregation. The breeders were astute enough to recognize this bull when he appeared with the right combination of genes. The Santa Gertrudis is based upon such happy results.

I agree with Hutt that if you examine a large number of strains (he has an advantage in dealing with poultry that breed more rapidly than cattle) you can choose the good ones and capitalize on these; but my point is that we cannot depend upon a happy accident. We should use this kind of accident when it arises, but we cannot relax and wait for it.

Until we know why these things occur, and until we are certain of the nature of the individual energy transfer processes, we cannot feel that we are doing our best.

Reverting to the White Leghorns: my interest in poultry arose many years ago when we had an exceptional heat wave in Brisbane, Australia, and lost a large number of fowls in one day.

It is accepted among poultry farmers that White Leghorns can withstand high or hot conditions much better than Australorps. Farmers were satisfied that they knew why: White Leghorns are white and Australorps are black; anybody knows that it is unwise to wear black clothing on a hot day.

We knew this explanation was unsatisfactory because the subject animals were in the shade, and there the animal's color makes no difference. Both white and black animals behave essentially as black bodies toward infrared. As scientists, we speculated: the reason for the superiority of the White Leghorns was their large wattles and combs, whereas the Australorps had only a small amount of tissue hardly recognizable as such.

To demonstrate our theory, we cut off the combs and wattles from the White Leghorns; it did not make any difference.

Incidentally, we have done that since with the big dewlap in Zebu cattle. Again it did not make any difference. This breed retained exactly the same tolerance toward heat as the other animals that we had previously tested.

HOWARD LEVENE (*Department of Mathematical Statistics and Zoology, Columbia University, New York, N.Y.*): Lee states that when he considered pure breeds of various animals together with physiological characteristics he found marked differences between individuals, suggesting that it is useless to choose a breed because of its heat resistance.

I suggest that breeders who have pure breeds probably overestimate the purity; this is also true of breeders who have inbred laboratory strains of various experimental animals. One inbreeds these animals in an attempt to make them homozygous and uniform, whether using a stud book or laboratory records. The difficulty is, as Wallace points out, that there is considerable evidence that animals that are heterozygous for a large number of loci are superior. Wallace comments particularly about blood group loci in chickens. In this instance, you have opposing factors: although you are inbreeding, there is natural selection, unknown to you, choosing for heterozygosity or variability at a great many loci.

With respect to pure breeds, there are certain traits that are represented in the breed itself. These may be a red coat color, a certain conformation, or others. A breeder selects for these traits and fixes them fairly well; but there are things that the breeder might overlook: he might overlook a physiological trait and consequently not fix it.

Nevertheless, once he recognizes such a trait as important, he can produce a subbreed by breeding for heat resistance or for some other physiological trait, and then produce a pure breed that will be uniform for the particular physiological trait desired.

I suggest that it is a good idea, while breeding for traits, not to worry if the animals produced have different coat colors. The more traits one tries to fix, the harder the task becomes.

I am interested in Newman's comment that Mongoloid people have adapted themselves to the tropics, cold places, and high altitudes, whereas the whites, relatively speaking, have been less adapted to various extremes.

There are two ways in which people can be adapted to extremes: (1) through a phenotypic plasticity, that is, a genetic make-up that develops physiological traits enabling one to adapt fairly easily to extremes in environmental conditions; and (2) through a genetic adaptation in which a person develops various genes that are advantageous under the particular conditions involved. The question then arises as to what is involved in the case of the Mongoloid. If we examine the Mongoloids' geographic distribution, we shall see they are the races (if we may use the word "race") present in South America and in the neighborhood of the Himalayas. These are places where it is possible for men to live on high plateaus. It was the Mongoloids who ventured there and who perhaps ultimately will be able to take over through the process of natural selection. On the other hand, the white race inhabited Europe primarily where there were mountains but not high ones. A person cannot live on a high mountain, except perhaps in Switzerland, where mountains are not as steep as in other places. In Europe, there were not the plateau regions where this kind of adaptation could take place.

Also, the whites were not in predominantly tropical places such as Africa, Central America, North and South America, and parts of Asia.

I wonder if Newman thinks this wide range of the Mongoloids is primarily due to the fact that one group (Eskimos) is adapted to cold, two different groups (the Tibetans and the Andes Indians) are adapted to high climates, and other groups to warm; or whether there is some indication that Mongoloids are more adaptable to extremes than whites.

NEWMAN: Primarily, full-blown Mongoloids with all their specialized features, such as fat padding around the face, body build, long trunk, short legs, and others, are cold-adapted people. Others traditionally considered Mongoloid may lack all these specializations in a fully developed form.

The altitude adaptation of Mongoloids in Tibet and the Andes mountains can be a matter of circumstance. One of my colleagues, Carl Coon, has observed that mysteriously, perhaps not accidentally, Mongoloid peoples are the only high-altitude dwellers today. Perhaps this trait is a historical accident of ethnic distribution. Your argument would be strengthened if you asked me what a Mongoloid really is, because Andean Indians do not fully fit the description.

Relative to the whites, they have pre-empted the most temperate lands and have lived on them for a long time. Price (cited elsewhere in this monograph) has presented evidence that without excessive mother-country coddling in the wet tropical forests the whites would not have thrived particularly well.

Although this field is incompletely known, there appear to be certain extra stresses put on whites. For example, in the lesser Antilles, where the French have tried to settle, the whites have been unable to compete in reproduction. This has been the trend, as Price points out.

How much of these specialized adjustments are adaptations in a genetic sense? How much is plasticity? As I have indicated elsewhere in this monograph, I do not know. I prefer to dodge these questions by relying on Hulse's suggestion that plasticity responses are genetically based. There is a tremendous individual range and also a considerable population range.

LEVENE: I ask Newman whether there have been studies on what are called typical Mongoloids, that is, the Chinese or Japanese—certainly not Eskimos—determining how these people who possess this cold adaptation react in tropical situations.

NEWMAN: Whites adapt themselves well, it seems, in desert heat. Arabs are a classically Mediterranean white. They do well there also, with cultural paraphernalia and know-how. They are a prime example of the linearity of body build that appears in desert-living men.

I know of no studies in this field other than that of Adam *et al.* (cited elsewhere in this monograph). As pointed out there, Asians showed somewhat lower sweating rates and presumably, maintained thermal balance a little better than acclimated British, that is, the British presumably living in Asia for a long time, or even born there, under conditions of humid heat.

As to what would happen if we took full-blown Mongoloid Buriats of the Baikal region of Siberia and relocated them in a tropical area in Western Oceania, I do not know. I suggest that Frederick Sergeant's hypothesis clearly implies that those who can survive in one extreme perhaps can survive in others.

Other than the evidence that I have mentioned, I have no direct evidence that Mongoloids survive well in tropics; however, they are certainly there and are thriving. Also, there are the so-called Malay people that descended into Oceania and are doing well there. Perhaps proof of the pudding lies in the tasting.

MANUEL M. VILLAVERDE (*Hospital Mercedes, Havana, Cuba*): After 8 years as a physician in Madrid, Spain, where conditions are similar to those in the United States, and 23 years thereafter in Havana, Cuba, a tropical country, I was impressed with some differences between what I saw in Spain and in Cuba. One thing that impressed me involved cholesterol, discussed by Osborne. The cholesterol level in Cuba is higher than that in most countries. The average in most countries ranges from 100 to 300 mg., according to some authors, and a mean of 145 to 175 mg., according to others. I found a mean figure in Cuba of approximately 186 (175 to 200 mg.).

My studies on seasonal variations of cholesterol disclosed that during the summer the cholesterol level is a little higher than in wintertime. It only includes that of white people living in tropical countries. Variations occurred in Europeans or descendants from Europeans.

With respect to body type, I found that most people in Cuba correspond to the asthenic type (tall and thin in figure). A percentage of 59.7 of the Cuban population corresponds to the asthenic type, and only a percentage of 13.7 corresponds to the pyknic type (small and thick in figure).

In regard to metabolism: I found it ranged from −15 per cent to +5 per cent in 1000 cases. In the United States, it is from 4 to 8 per cent higher than in Cuba.

In my studies of blood calcium I found the data in Cuba and in the United States to be the same. The values ranged from 9 to 11 mg., but there were a larger number of cases presenting higher than lower values. In other words, there were more cases over 10 mg. per cent than under 10 mg. per cent.

Finally, regarding nutrition, a study was made by United States physicians on vitamin B_1, or thiamine, and they agreed that there was a slight deficiency among children in Cuba. I disagree because of my personal experiences while practicing in Cuba.

KURT HIRSCHHORN (*New York University Medical Center, New York, N.Y.*): I want to make two brief comments. First, going back to cholesterol: I suggest, as to the difference between the two studies mentioned by Osborne, that it is fairly well established that variation in cholesterol can be determined, and usually is determined, by a primary variation in the neutral fat or triglyceride level of the blood. This is true of the variation with age, that is, the increase of cholesterol with age. Also, it is apparently true for the increase in cholesterol with coronary artery disease.

With respect to triglyceride in the blood, it is varied both by heredity and by environment. There are several genetically determined conditions that delay the clearing of ingested fat and produce first an elevation of triglyceride, and then a secondary elevation of cholesterol. By increasing their fat intake, most people probably can increase their triglyceride as well as their cholesterol levels.

Therefore I believe that if triglycerides were to be measured in the two studies mentioned by Osborne, not only would the differences between the studies be accentuated but Osborne's own data would better show the statistical differences in those categories that he has measured.

My second comment relates to the relatively low incidence of scurvy among the Bantus, despite a low vitamin C intake. The inability to make ascorbic acid by man, anthropoids, and guinea pigs has been called an inborn error of metabolism, since all other species appear able to do this.

Since this defect in the guinea pig seems totally different from that in anthropoids and since history discloses that not all British sailors became inflicted with scurvy and not all Bantus contract the disease, I question whether this is not closer to what we usually consider an inborn error of metabolism and whether there is variation within human populations. It may be that a certain number of humans are able to produce ascorbic acid or, at least, do not destroy it as quickly as is thought.

As this defect is apparently a widely distributed one, I am uncertain what population studies for the defect might disclose.

DAVID YI-YUNG HSIA (*Children's Memorial Hospital, Chicago, Ill.*): I add one other concept that probably more properly belongs in the inborn errors of metabolism.

We all know that cystic fibrosis of the pancreas is one of the most commonly known genetic errors of metabolism in white populations. It is interesting that one of the major defects in cystic fibrosis is the fact that children with this disease possess exceptionally high electrolyte content of sodium and chloride in their sweat. In fact, this content approximates 4 to 5 times that normally seen in normal children or adults.

Actually, this sweat defect was first discovered in New York, N.Y., during a heat wave when a large number of the cystic fibrotic children at Babies Hospital in that city were admitted in a state of heat prostration; practically none of a much larger group of patients without cystic fibrosis experienced heat prostration during this same heat wave.

This fact immediately identified the children as different from the random population. Although in New York heat prostration could have been quickly treated, prompt treatment probably would not have been possible under primitive conditions in Africa, and these children undoubtedly would have died.

This brings up the interesting question of why this condition is almost never seen in Negroes, and why it occurs at a frequency of approximately 1 to 2000 births among whites.

For instance, in our own clinic we have approximately 140 white families with cystic fibrosis and only two Negro families, although admissions in our hospital run well over one-half Negro. Certainly this suggests that there is some interacting force going on in this situation.

Reference

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Part II. Genetic Errors of Metabolism and Environmental Interaction

HERITABLE DISORDERS OF ACID MUCOPOLYSACCHARIDE METABOLISM IN HUMANS AND IN SNORTER DWARF CATTLE*

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As increasing investigative effort has been applied to the study of connective tissues, recognition of multiple diverse physiological functions and increased knowledge concerning the physical and chemical constitution of these tissues have been effected.[1-5] Connective tissue may be broadly defined as the barrier that exists between the circulation and the parenchymal cells of the body. It is composed essentially of three major types of elements, namely (1) specialized connective tissue cellular elements (for example, fibroblasts, mast cells, chondroblasts, and osteoblasts); (2) the structured collagenous and elastic fibers; and (3) the extracellular, extrafibrillar amorphous matrix or ground substance in which these cellular and fibrillar elements are embedded. The relative amounts of these elements together with the qualitative differences in the chemical composition of the ground substance determine the varied properties and specific functions of the different connective tissues throughout the body. Connective tissue ranges from one extreme of gel-like consistency such as is present in the Wharton's jelly of the umbilical cord, the vitreous humor, and synovial fluid to the other extreme of tissue density that is present in tendon, cartilage, and bone.

The studies presented here are primarily concerned with those heritable disorders in man and cattle in which manifestations of altered connective tissue acid mucopolysaccharide metabolism have been demonstrated. The connective tissue acid mucopolysaccharides complexed with proteins are chemical constituents present in, and for the most part unique to, the ground substance. They are large, linear carbohydrate polymers that can be separated from their protein component by relatively mild chemical procedures.

These mucopolysaccharides are polymers of high molecular weight, and are made up of repeating disaccharide units, which are most frequently composed of a hexosamine and a hexuronic acid moiety joined by a glycosidic bond (keratosulfate does not contain a uronic acid moiety; the hexosamine is linked to the hexose galactose). Frequently, as in hyaluronic acid (HA) and in the three chondroitin sulfuric acids (CSA-A, CSA-B, and CSA-C), the amino group of the hexosamine is acetylated. In others, such as heparin, heparin monosulfuric acid, and the chondroitin sulfuric acids, variable amounts of sulfate are present.

Currently about eight distinct connective tissue acid mucopolysaccharides are known, and some information of their distribution in tissues has been determined.[3-5] It seems reasonable to assume that further studies of connec-

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tive tissue will reveal the presence of additional mucopolysaccharides that as yet have not been characterized.

It should be noted that the isolation and characterization of these complex polymers are difficult tasks since the techniques currently available make it difficult to determine the homogeneity of an isolated fraction, not to mention any changes that might be brought about by the methods used to isolate the mucopolysaccharide components from various biological tissues.

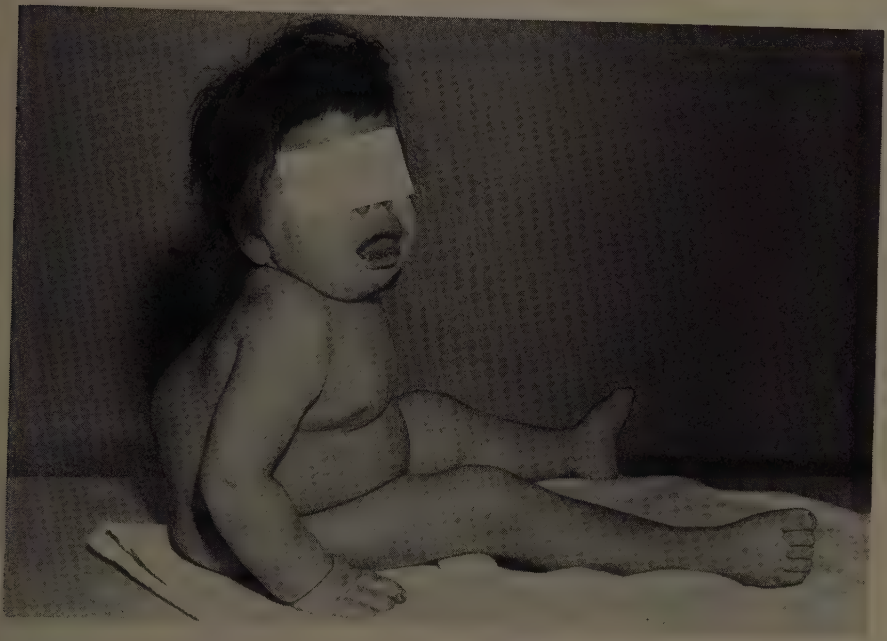

FIGURE 1. Seven-year-old girl with typical appearance of fully developed Hurler's syndrome.

The Hurler Syndrome

With Brante's[6] finding that the "storage material" found in autopsy specimens obtained from patients with the Hurler syndrome (lipochondrodystrophy, gargoylism) is acid mucopolysaccharide in nature came the recognition of the first such disorder in humans that appeared to represent a hereditary defect involving the metabolism of these compounds. Dorfman and Lorincz[7,8] and Meyer and his associates[9,10] later demonstrated that large amounts of chondroitin sulfuric acid-B (CSA-B or β-heparin) and a heparin monosulfuric acid-like polysaccharide (heparitin) are found in the urine and tissues of these individuals, offering further corroboration of the fact that this disorder is a mucopolysaccharidosis.

Apparently related to this derangement in mucopolysaccharide metabolism, the skeleton in the Hurler syndrome is progressively malformed in a rather characteristic (FIGURE 1) grotesque manner that results in a disproportionately large skull, short neck, dwarfed stature, flexion contractures that affect the upper extremities to a greater extent than the lower extremities, and a kyphosis of the thoracolumbar spine. A protuberant abdomen with enlargement of the liver and spleen, diastasis recti with umbilical and inguinal herniation are also common in this disorder; characteristic coarse facial features, square broad hands with short, stubby, tapering fingers fixed in partial flexion are also common results of this condition. At birth the affected individuals are fairly normal in appearance, and all of the above changes develop and progress with increasing age. Most affected individuals die before the second decade of life.[11]

At autopsy, large vacuolated cells are frequently seen in many tissues, but they are most common in the liver, spleen, and brain. Heavy deposits in the intima of the large blood vessels and arteries frequently lead to angina pectoris and sudden death. Interestingly, these changes are seen in children who may be only a few years of age. Associated with the blood vessel changes are nodular fibrous thickenings of the heart valves, which grossly resemble changes of far-advanced valvular rheumatic heart disease.

Progressive impairment of the intellect is also a feature of this syndrome. The meninges become thickened with excessive deposition of mucopolysaccharides. Internal hydrocephalus occurs in many cases. Progressive, diffuse clouding of the cornea is seen in many instances.

Genetic Transmission of the Hurler Syndrome

There are two genetic varieties of the Hurler syndrome. The most common form of the disease is autosomal recessive, affecting males and females in approximately equal frequency and tending to show an increased rate of consanguinity among the parents. A large percentage of subjects with this form of the disorder will show corneal clouding. The second variety of genetic transmission is as a sex-linked recessive, as in classic hemophilia. In this form of the disease, where only males are affected, corneal clouding is not seen, and the evolution of the disease is slower. Patients with this form are apt to live longer and are as a whole less severely affected.[12]

There are no well-documented descriptions of skeletal deformities in close relatives of patients with fully developed cases to suggest that partial expressions of the trait in the heterozygous state may occur. Parents invariably have been unaffected.

Biochemical Manifestations in the Hurler Syndrome

Biochemically, three variants of acid mucopolysaccharide excretion and accumulation in tissues have been shown to occur in this clinical syndrome: (1) one variant group excretes predominantly CSA-B; this is the mucopolysaccharide found in excessive amounts in the liver; (2) another group excretes predominantly the heparin monosulfuric acid-like polysaccharide with accumulation of this compound in tissues; and (3) the largest group excretes both of these acid mucopolysaccharides in varying proportions.[10,13,14]

To date no biochemical differences between the two genotypes, as to excretion pattern or tissue deposits, can be demonstrated. Whether this abnormal "storage" in tissues and excretion in urine of acid mucopolysaccharides (present normally in connective tissues but not in urine) represents excessive production or underutilization remains to be determined.

Multiple Exostoses

During 1960 I had the opportunity of studying three families with another heritable disorder that affects skeletal development, known as hereditary multiple exostoses or hereditary deforming chondrodysplasia, and known in England as diaphysial aclasis.[15] This abnormality of bone and cartilage development of unknown cause has been described in the medical literature for more than 100 years. Its inheritance follows the pattern of a dominant characteristic with incomplete penetrance in the female. Its incidence in males is approximately three times greater than in females. All bones of the body may be involved; however, the most common sites are the ends of the tubular long bones, as well as the ribs and the scapulae.

The exostoses apparently represent an aberrant plate of subperiosteal cartilage that continues to proliferate. Enchondral ossification then occurs in a manner similar to that seen at a normal epiphyseal line. The lesions vary in number, but as many as several hundred have been reported to occur in a single case. Histologically, a hyaline cartilage layer crowns each exostosis. Its periosteum, cortex, and cancellous trabeculations are continuous with these layers in the underlying bone. The cancellous trabeculations show variable degrees of distortion and irregularity. Growth continues in the exostoses until the time of closure of the epiphyses; thereafter renewed activity usually indicates a sarcomatous change.

Mucopolysacchariduria in Heritable Multiple Exostoses

Urinary acid mucopolysaccharide excretion was studied initially by using the acidified bovine serum albumin turbidity technique previously used as a screening test for detecting excessive mucopolysacchariduria in the Hurler syndrome.[14] The turbidity produced in unaffected growing children with multiple exostoses was definitely increased; occasionally it was observed to be as marked as in the Hurler syndrome. Affected adults, normal siblings, and relatives failed to demonstrate this gross quantitative increase in acid mucopolysaccharide excretion.

Actual isolation of the crude acid mucopolysaccharide fractions from urine was performed, using the method previously employed by Dorfman and Lorincz in their investigations of the Hurler syndrome.[8] With minor modifications, this method was employed in the urine and tissue studies presented here in both the human and the cattle investigations.

Further purification and fractionation of the crude mucopolysaccharide thus obtained was done by the method of Schiller and Slover,[16] which is dependent on the differential solubility of the cetyl pyridinium chloride-acid mucopolysaccharide complexes in varying concentrations of sodium chloride. With this method of fractionation, HA is found in the 0.4 M sodium chloride fraction; CSA-A, CSA-B, and CSA-C in the 1.2 M fraction, and regular heparin in the 2.1 M fraction.

The amount of crude mucopolysaccharide recovered from growing children affected with this disorder was in the order of magnitude of 10 to 20 mg./l.; the predominant amount of polysaccharide excreted was recovered in the 1.2 *M* sodium chloride-soluble fraction and was susceptible to complete digestion by testicular hyaluronidase. Chemical analyses for hexosamine,[17] uronic acid,[18] acetyl, nitrogen, and sulfate showed molar ratios of these substances similar to those reported for CSA-A and CSA-C. Infrared spectral analysis of this polysaccharide was similar to the infrared pattern reported for CSA-A.[19] Hydrolyzates of the polysaccharide revealed the presence of galactosamine and glucuronic acid. On the basis of these data, the identification of CSA-A as the major mucopolysaccharide excreted by affected growing children with hereditary multiple exostoses has been made.[20]

CSA-A is the mucopolysaccharide reported to be excreted in trace amounts by normal individuals.[21] Di Ferrante, Rich and his associates,[22] estimating total urinary mucopolysaccharide excretion on the basis of uronic acid content as determined by the carbazole method of Dische,[18] have reported increased excretion of CSA-A–like polysaccharides in normal children as well as in patients with certain neoplastic diseases[23] and lupus erythematosus.[24] Their estimated increase of urinary mucopolysacchariduria in these conditions does not approximate the quantitative amount of acid mucopolysaccharide recovered in the urine of either individuals with the Hurler syndrome or growing children with heritable multiple exostoses.

Hereditary Arthro-osteo-onychodysplasia

More recently, I had the opportunity of studying a single family with still another unusual hereditary disease in which there are changes in tissues that derive from both ectodermal and mesenchymal germ layers of the embryo, namely hereditary arthro-osteo-onychodysplasia. This syndrome has been described in the medical literature since the last part of the century[25] under such different names as osseous dysplasia and dystrophy of the nails,[26] osteo-onychodysplasia,[27] and Turner's syndrome.[28] More findings have been added, and a better explanation of the genetic transmission has been introduced in the recent literature.[29]

The following list of findings has been described in this syndrome; it is presented approximately in the order of frequency of occurrence: (1) dysplasias of the pelvic girdle, including iliac horns, hypoplasia of the iliac bones, protrusion of the acetabulum, and coxa valga; (2) dysplasias of the knee joints that involve the patella, the femoral condyles, and the head of the fibula; (3) dysplasias of the nails; (4) abnormalities of pigmentation of the iris; (5) dysplasias of the elbow, foot, arm, and hand; (6) dysplasia of the shoulder girdle; and (7) hyperostosis frontalis of the skull.

In most cases these deformities do not produce symptoms, and the patients adapt themselves quite well to the demands of normal activities. Rarely do they seek medical care because of these defects; most commonly the diagnosis is made as an incidental finding.

Arthro-osteo-onychodysplasia is transmitted as an autosomal dominant trait.

Renwick and Lawler[30] have concluded that there is a significant relationship between the ABO blood groups and this syndrome. No linkage was demonstrated with other blood group loci.

Mucopolysacchariduria in Hereditary Arthro-osteo-onychodysplasia

The family that I have had the opportunity to study has been reported by Papadimitriou.[29] Three affected children, ages 18 months, 5 years, and 10 years, and the affected paternal grandmother were available for study. This disorder was reported to have occurred in 28 of the 58 known members of this family over 5 generations.

All 3 of the affected children had a marked increase in urinary acid-mucopolysaccharide excretion as estimated by the acid albumin turbidity screening technique. A normal 3-year-old sibling, the mother, and the affected grandmother did not show this increased turbidity.

Appropriate isolation and preliminary identification of the mucopolysaccharide excreted by the 2 older affected girls revealed the major amount of polysaccharide excreted to be present in the 0.4 M sodium chloride-soluble fraction. This polysaccharide is not yet completely characterized; however, on the basis of the cetyl pyridinium chloride fractionation, susceptibility to complete digestion by testicular hyaluronidase, migration, and staining characteristics on paper electrophoresis, and on the finding of approximate equimolar ratios of uronic acid and hexosamine, the polysaccharide appears to be similar to HA.

The number of such cases studied is still too small to draw any conclusions as to whether mucopolysacchariduria is uniformly present in all such affected children and absent in affected adults. It may be postulated that the dysplasias of the joints may be related to the faulty or altered metabolism of synovial fluid HA-protein complexes. This is at best purely speculative; it would not explain at the present time the dysplastic changes found in the nails.

From these data there appear to be at least three known human heritable disorders that have demonstrable alterations in connective tissue acid mucopolysaccharide metabolism. Preliminary reports by Berenson and Serra[31] suggest the occurrence of increased mucopolysacchariduria in the Marfan syndrome. This would represent the fourth such disorder in humans. There is every reason to believe that other such disorders will soon be recognized as appropriate studies are advanced.

Altered Acid Mucopolysaccharide Metabolism in Cattle

Generalized disorders of connective tissue acid mucopolysaccharide metabolism seem to have been completely unknown in animals. That the existence of such disorders in animals would be found had been anticipated by many.

Recently I had the good fortune of becoming associated with Marvin Koger of the Department of Animal Husbandry of the University of Florida at Gainesville, Fla. He and his colleagues have been studying the genetic, anatomical, and physiological aspects of dwarfism in cattle.[32-34] A variety of dwarf cattle of different types and breeds are available for study. Among these animals are a group known as snorter dwarfs or brachycephalic dwarf cattle.

Snorter Dwarf Cattle

This particular type of dwarfism in cattle is heritable. Available genetic data indicates it is transmitted as an autosomal recessive. It is known to occur in many breeds of cattle; currently it represents a major problem to Hereford and Angus cattle breeders. Characteristic of these dwarf animals is the heavy labored breathing from which the name "snorter" is derived. The eyes are widely spaced; the nose is short; and the mandible is longer than the maxilla so that malocclusion of the incisors with the dental pad is often present (FIGURE 2). The head is short, broad, and low set; the animal in general is compact and blocky with a shorter neck and more width through the body than in a normal animal. These dwarf animals have an overly mature appearance when young. Shortened long bones and shortened, beaded lumbar vertebrae as well as a bulging forehead with demonstrable premature closure of the spheno-occipital synchondrosis are common characteristics.[35,36] Internal hydrocephalus, enlarged tongue, rough thickened skin, and abdominal hernias are also often present. At birth these calves appear fairly normal (FIGURE 3); by 2 to 4 months of age the dwarfs appear to be stunted and potbellied (FIGURE 4). Most of these animals attain a certain level of development and then regress; many die before the age of 1 year. In the normal course of events cattlemen usually destroy the snorter dwarfs as soon as they are recognized as such.

FIGURE 2. Head of a 2-year-old Angus snorter bull.

The first scientific description of these animals reportedly was made by Johnson and his co-workers in 1950.[37] In Charles Darwin's "Voyage of the Beagle"[38] the following description of a herd of cattle is recorded in his diary dated November 18, 1832.

". . . at the Arroyo de San Juan . . . on two occasions I met with, in this province, some oxen of a curious breed, called nata or niata. They appear externally to hold nearly the same relation to other cattle, which bull or pug dogs do to other dogs. Their forehead is very short and broad, with the nasal end turned up, and the upper lip much drawn back; their lower jaws project beyond the upper and have a corresponding upward curve; seated high up and very

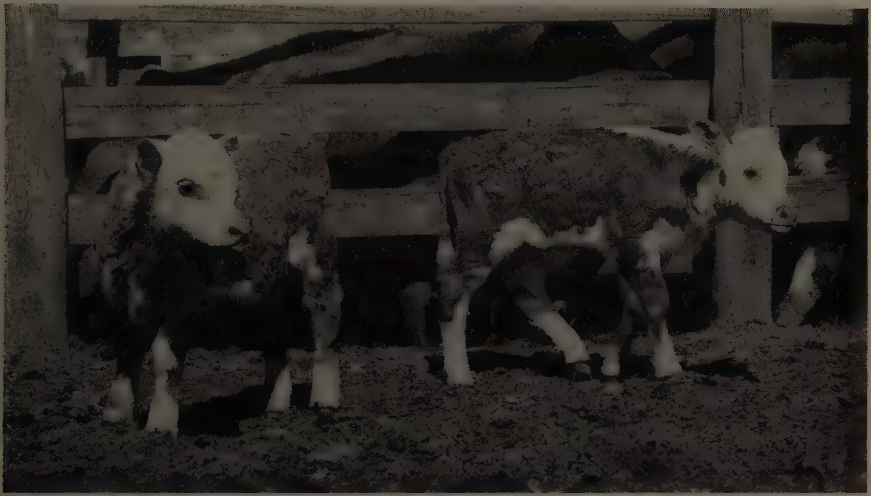

FIGURE 3. Animal on the left is a 1-month-old Hereford snorter calf. The animal on the right is a 6-week-old unaffected Hereford calf.

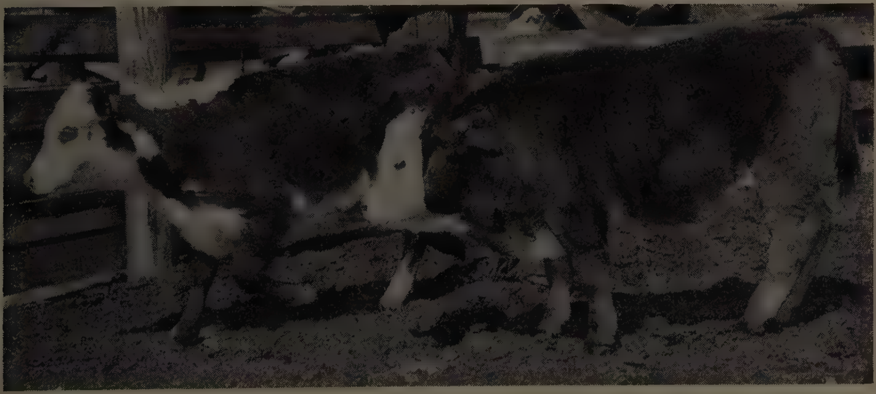

FIGURE 4. Four-month-old Hereford snorter in foreground (*right*) with unaffected Hereford of the same age (*left*).

open, their eyes project outwards. When walking they carry their heads low, on a short neck; and their hinder legs are rather longer compared with the front legs than is usual. Their bare teeth, their short heads, and upturned nostrils give them the most ludicrous self-confident air of defiance imaginable" (FIGURE 5). ". . . during the great droughts, when so many animals perish, the niata breed is under a great disadvantage, and would be exterminated if not attended to; for the common cattle, like horses, are able to keep just alive, by browsing with their lips on twigs of trees and reeds; this the niatas cannot do, as their lips do not join, and hence they are found to perish before the common cattle."

Whether these niata cattle described by Darwin were truly snorter dwarf cattle is difficult to determine, but at least it seems appropriate to bring to light this observation and description in a monograph concerned with heredity and environmental interaction.

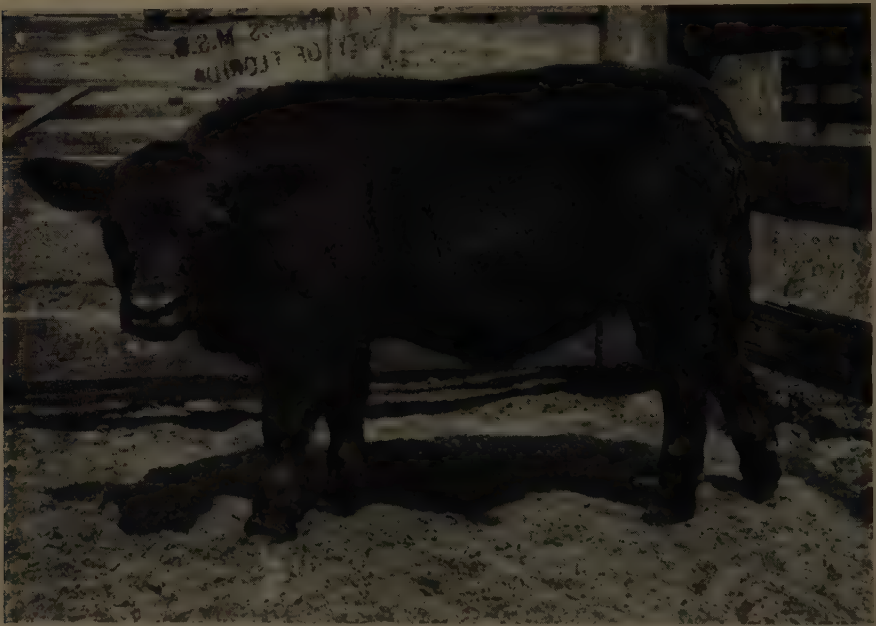

FIGURE 5. Six-month-old Angus snorter calf.

There has been a marked increase in the frequency of the appearance of this trait in the past decade. Presumably, as livestock-judging standards have changed to select for shorter-legged, blocked-up, compact, short-faced animals, a greater number of carriers have inadvertently been selected for breeding purposes.

Altered Mucopolysaccharide Metabolism in Snorter Dwarf Cattle

The characteristics of the snorter dwarf cattle appeared so analogous to the human form of dwarfism known as the Hurler syndrome that the logical next step was a search for evidence of abnormal acid mucopolysaccharide metabolism in these animals. Urinary acid mucopolysaccharide excretion in snorter dwarfs, known carriers of the trait, and in normal, presumed unaffected cattle was initially studied by means of a slightly modified acid albumin turbidity screening technique. Aliquots from 24-hour urine collections of these animals were obtained and dialyzed. The turbidity had to be corrected for intense coloring of many of the urine specimens. An optical density reading of 0.040 at a wave length of 600 mμ as read on a Coleman Junior Spectrophotometer was selected as the upper limit of normal. Nine of 9 snorter dwarf animals had abnormally elevated turbidity values. One of these 9 animals, a 2-year-old Angus bull, on 3 occasions excreted urine that tested in the high normal range. Sixteen presumably unaffected cows, which included midget Brahmans, Florida Guinea cattle, and several normal dairy breeds, were used to establish the "normal" levels of excretion as estimated by the turbidity technique.

More than 40 casual urine specimens obtained from phenotypically normal animals gave optical density readings well below 0.040, the great majority falling below 0.020. To date the only "false positive values" employing the above criteria have been 24-hour collections into which vaginal mucus seeped into the collection. In obtaining 24-hour collections from cows, the rubber damming that was glued around the vagina occasionally caused irritation and excessive mucus discharge. Subsequent testing of urine samples that were not contaminated with mucus, tested in the normal range.

Four known carriers of the snorter dwarf trait, which phenotypically appeared normal, gave readings in the normal range. Obviously these numbers are too small to establish any truly representative normal values of cattle urinary mucopolysaccharide excretion, especially when such factors as breed, age, sex, nutrition, state of health, and activity also must be taken into account.

Recovery of crude mucopolysaccharide from presumably normal and carrier cows was less than 5 mg./l., whereas the snorter dwarf cattle excreted 25 to 50 mg./l. Two major polysaccharide components have been recovered on fractionation of the crude mucopolysaccharide obtained from affected cattle; one appears to be similar to CSA-B and is recovered in the 1.2 *M* sodium chloride soluble fraction; the other has properties similar to HA and is recovered in the 0.4 *M* fraction.

To date, tissues from 3 affected animals have been available for study. One was a calf that phenotypically appeared normal at birth when delivered by Caesarean section of a snorter Angus cow. This calf died of an undetermined cause within 2 hours of birth. The liver was somewhat small and abnormally yellowish in color. The calf had evidence of mild internal hydrocephalus and showed some early tendency to premature closure of the spheno-occipital synchondrosis, a change that is normally seen only at 24 to 36 months of age.[36]

The second animal was a stillborn calf (FIGURE 6) with the extreme form of the snorter trait that was born to a known Hereford carrier of this disorder.

The third animal (FIGURE 7) whose tissues were available for study was a 2-year-old Angus bull that had to be sacrificed because of evidences of an impending, progressive terminal course. Examination of the heart revealed the presence of cardiac hypertrophy and fibrous nodular thickening of the mitral valves (FIGURE 8).

Control normal livers from unaffected newborn calves and adult cows were

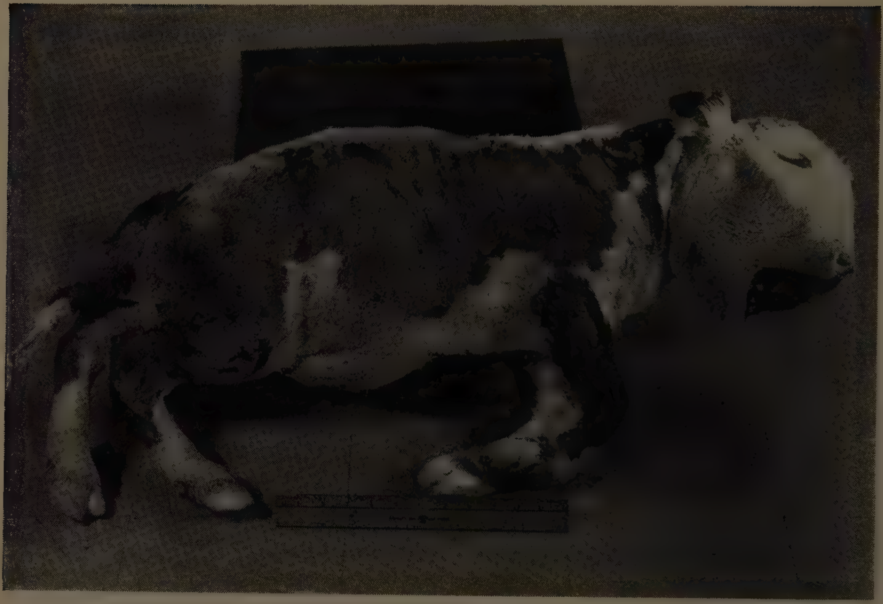

FIGURE 6. Stillborn Hereford calf with "extreme" form of snorter dwarf trait.

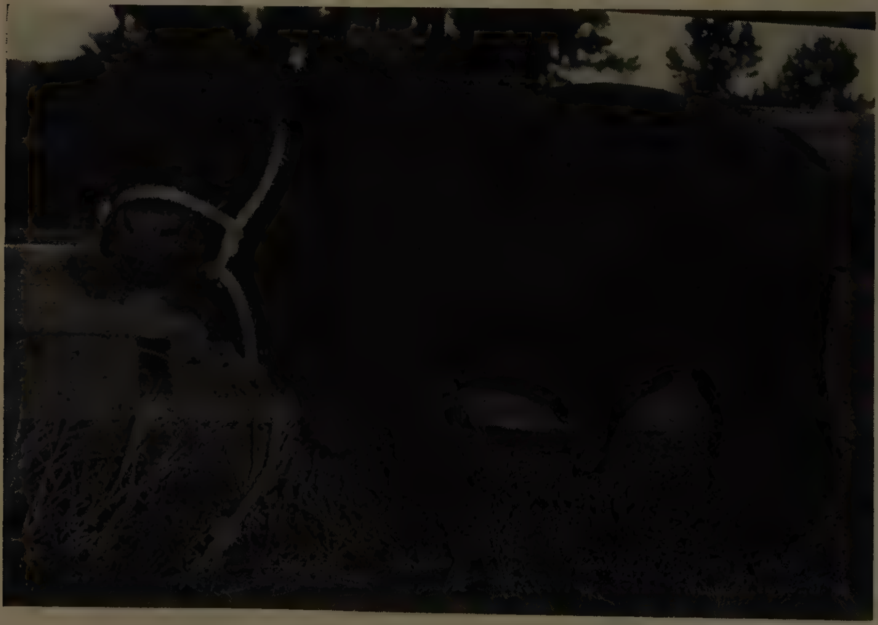

FIGURE 7. Two-year-old Angus snorter bull.

found to contain only minimal amounts of cetyl pyridinium chloride, precipitable, nondialyzable, uronic acid-containing polysaccharide. Most of this polysaccharide from normal livers appeared in the 2.1 *M* sodium chloride soluble fraction.

In the 3 affected animals, 1 to 2 per cent of the dry liver acetone powder was found to be acid mucopolysaccharide in nature. Most of the liver polysaccharide obtained from the calf delivered by Caesarean section was recovered in the 0.4 *M* sodium chloride soluble fraction and was completely susceptible to digestion by testicular hyaluronidase; it also had staining characteristics with toluidine blue and mobility on paper electrophoresis similar to that of HA.

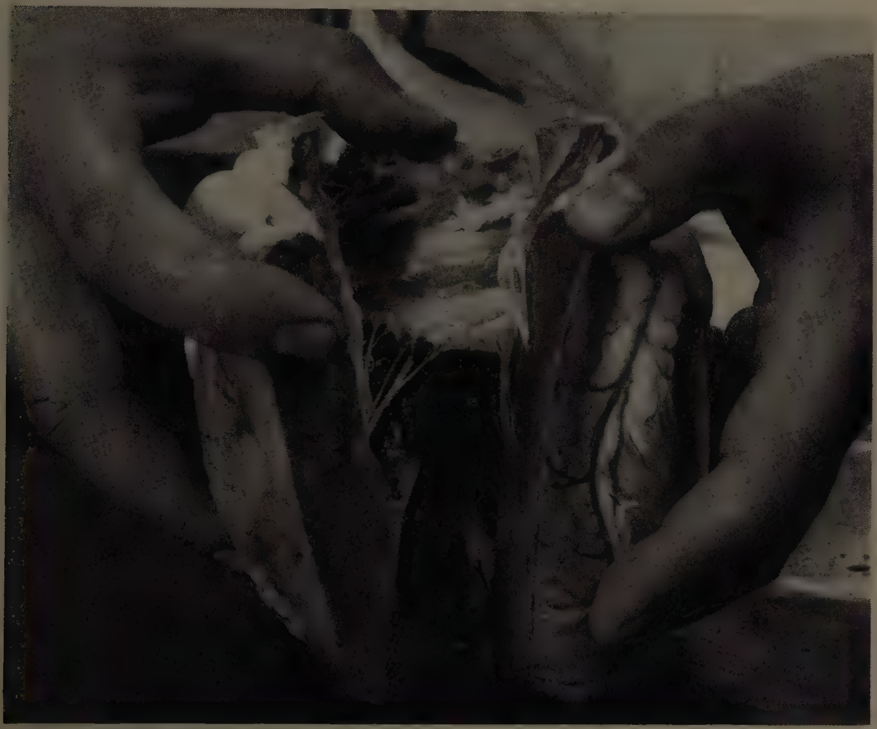

FIGURE 8. Close-up view of mitral valve of the animal shown in FIGURE 7, showing rough, nodular thickening. Visible in the left auricle is a fibrous band believed to be the result of "jet lesion" of mitral insufficiency.

The liver from the stillborn "extreme" calf had 2 major acid mucopolysaccharide components: one similar to that found in the liver of the first animal studied, which appeared to be similar to HA, the other, recovered in the 1.2 *M* fraction, which had the mobility of one of the chondroitin sulfuric acids on paper electrophoresis and was resistant to digestion by testicular hyaluronidase. Tentatively, prior to more definite characterization, this latter component appears to be similar to CSA-B, the same mucopolysaccharide that is accumulated in tissues and excreted in the urine of a large number of individuals having the Hurler syndrome.

More interestingly, the same 2 major components were found in the liver of the 2-year-old Angus bull. The urine from this bull was found to have what appears to be the identical 2 acid mucopolysaccharide components found in its liver. This correlation between the acid mucopolysaccharide present in tissues with the substance excreted in urine again parallels the situation observed in the Hurler syndrome.[13]

More definitive characterization of these acid mucopolysaccharides, which appear in excessive amounts in the urine and tissues of these snorter dwarf cattle, remain to be completed. If the polysaccharide recovered in the 0.4 M sodium chloride fraction is found to be HA, it is the first time to the best of my knowledge that HA has been reported to occur in such amounts in the liver of any mammalian organism, including man.

Currently only sparse information is available on the intermediary metabolism and synthesis of the acid mucopolysaccharides per se.[2,4,5,39-41] The effects of such factors as heredity, hormones,[42,43] age, and nutrition on mucopolysaccharide metabolism are in evidence, but the basic mechanisms involved are not understood. What are the presumed enzymatic defects in the human heritable disorders such as the Hurler syndrome, hereditary multiple exostoses, and hereditary arthro-osteo-onychodysplasia? The answers to this question at the present time are not known. With the availability of a natural experimental animal model for the study of such altered acid mucopolysaccharide metabolism, perhaps the elucidation of the normal and abnormal metabolic pathways of these substances can be hastened.

Acknowledgments

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GENETIC VARIATION IN THE UTILIZATION OF RIBOFLAVIN, THIAMINE, AND OTHER NUTRIENTS

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Introduction

A casual glance around any audience of the highest Primates will suggest that some animals utilize their nutrients more efficiently than others. While some of the more readily perceptible differences may result chiefly from variations in the caloric intake, it is true, nevertheless, that there are remarkable differences among us, as among other animals, in the degree to which different individuals thrive on the same amounts of the same diet.

Those who raise domestic animals are much concerned about the efficiency of conversion of feedstuffs to edible beef, pork, poultry, and other meat. I do not propose to delve into that field here but rather to discuss examples of genetic differences in a vertebrate animal in ability to utilize specific nutrients in the diet. We now have ample evidence in the domestic fowl, to which species these remarks will be almost exclusively confined, of genetic differences in the requirement or utilization of minerals, of amino acids, and of certain vitamins. To be sure, we who work with vertebrates can not hope to equal the extensive discoveries of genetic differences in the synthesis and metabolism of nutrients that have been found in simpler organisms such as Neurospora. Some of the differences that we do find in our vertebrates have not even been subjected to detailed genetical analysis; we must therefore be content in many cases with demonstrable differences between breeds in nutritional requirements. However, since a breed is merely the outward expression of a genotype, a difference between breeds, provided that it is consistently maintained, is clearly a difference between genotypes.

The fowl is a particularly suitable species for studies of this kind. For one thing, the chick is characterized by a rapid rate of growth after hatching. If the diet is inadequate, or if the chick is genetically unable to utilize that diet, such shortcomings are quickly recognized. Equally important is the fact that nutritional deficiencies interfering with the growth of the embryo can be easily detected because the embryo can be observed at any desired stage during its 21 days of incubation.

Minerals

To the best of my knowledge, the first indication of genetic differences in nutritional requirements in the fowl was the discovery by Serfontein and Payne (1934) that White Leghorns are much more resistant than Rhode Island Reds to perosis, or "slipped tendon," a condition in which the intertarsal joint becomes enlarged and sometimes the gastrocnemius tendon slips out of the intercondylar groove. On a diet conducive to the development of that disease, when 14 per cent of the Rhode Island Reds came down with perosis, the corresponding incidence in White Leghorns on the same diet was only 0.7 per cent. A single generation of mass selection in the susceptible breed raised the proportion of affected birds to 50 per cent.

Serfontein and Payne did not know at the time that they were dealing with differences in the requirement of manganese, but this was demonstrated to be the case shortly after by Wilgus *et al.* (1937). Subsequent studies showed that chicks of heavy breeds may need as much as 50 parts per million (ppm) of manganese to ensure normal development of bone, whereas White Leghorns very rarely get perosis even on diets containing as little as 30 ppm of manganese.

Other evidence of a genetic basis for this difference in manganese requirement was found in an interesting way by Rosenberg and Tanaka (1951) who imported to Hawaii three lots of New Hampshire chicks: one from California, another from Washington, and the third from Oregon. By 12 weeks of age the proportions of the imported birds showing perosis varied from 3.9 to 5.2 per cent, but among New Hampshires reared concurrently from stock that had been bred in Hawaii for several years, not a single case appeared. The probable reason for the difference is very interesting. In continental United States most New Hampshires are raised on the flat floors of brooder houses, but in Hawaii, to avoid parasitism, which is extreme there, practically every chicken is raised in pens with wire floors. This latter practice is conducive to the aggravation of perosis in susceptible birds. It seems probable, therefore, that in Hawaii natural selection on the wire floors over a period of years had eliminated the genetically susceptible birds and produced a fairly resistant strain. Under brooding conditions with less stress in the United States there had not been the same degree of elimination of genetically susceptible stock.

It is fairly certain that there are genetic differences among the breeds and strains of the fowl in requirement of other minerals or in their ability to utilize them. Evidence has been reviewed elsewhere (Hutt, 1949) showing that White Leghorns tend to lay eggs with stronger shells than those produced by the heavy breeds and that by selection within a breed two distinct lines can be differentiated, the one characterized by comparatively thick shells, the other by thin ones. Presumably the difference depends in large part upon the utilization of calcium.

Amino Acids

While many studies have been made of the protein requirements of the fowl at different stages of its life, very few of these have been of such a nature as to reveal genetic differences in requirement of specific amino acids. However, McDonald (1957) has recently shown that Australorps, an Australian version of Black Orpingtons, are less able than White Leghorns to utilize methionine. Comparing chicks on a basal diet (deficient in methionine) with those given the same diet supplemented with 0.10 per cent DL-methionine, the response (measured by weight at 4 weeks of age) was about 10.9 per cent for the White Leghorns against 2.0 per cent for the Australorps, the difference being highly significant. When reciprocal crosses were made between the two breeds, female chicks sired by the Australorp male did not utilize methionine as well as the female chicks sired by the Leghorn. McDonald interpreted these results to mean that the utilization of methionine is influenced by a sex-linked gene or genes. In a later report (1958) he postulated that such a gene is linked with the gene *K*, which causes slow feathering, and thus possibly conserves cysteine. However, since no actual linkage data have been presented, and especially since there was considerable variation among the males in the progeny of McDonald's reciprocal crosses, it is desirable that his results be confirmed and extended before definite conclusions be drawn about a sex-linked gene that prevents the Australorps from utilizing methionine as well as the Leghorns can do. It is also desirable to determine whether or not other heavy breeds show the same peculiarity as the Australorps.

In spite of these reservations, McDonald's experiments are commendable and noteworthy as the first attempts to find genetic differences among fowls in requirement of specific amino acids. It is to be hoped that others will follow.

Vitamins

Thiamine. It is appropriate to begin consideration of genetic differences in the utilization of vitamins with thiamine. We owe the first steps toward the discovery of that vitamin (earlier called vitamin B_1) in part to the Dutch physician, Christiaan Eijkman, but also to the fowls fed on the garbage of his prison hospital in Java. These developed symptoms of paralysis and polyneuritis that Eijkman recognized as comparable to those of his patients afflicted with beriberi. The subsequent discoveries that polished rice lacks a nutrient essential for the prevention of beriberi and that rice with the hull left on contains that nutrient, which we now know as thiamine, are important milestones in the advancement of our knowledge of nutrition.

The geneticist, however, might well wonder what kind of fowls those were, for it has been demonstrated that breeds differ in requirement of thiamine and that White Leghorns are much more resistant to a deficiency of it than are heavy breeds such as Rhode Island Reds and Plymouth Rocks. The history of these studies has recently been reviewed elsewhere (Hutt, 1958), so it should suffice here to give only a brief summary. Adult birds of heavy breeds, such as Plymouth Rocks and Rhode Island Reds, are not likely to survive longer than three weeks on a diet that is markedly deficient in thiamine. In contrast, Nichita and Iftimesco (1934) found that White Leghorns could stand such diets for several months or more without showing any sign of polyneuritis. A similar difference between Leghorn chicks and those of heavy breeds was found by Lamoreux and Hutt (1939). Whether the chicks were started on a deficient diet at hatching, at two weeks, or at three weeks of age, in all cases the White Leghorns survived better than the heavy breeds.

Other evidence of more efficient utilization of thiamine by White Leghorns is found in the fact that they deposit more of that vitamin in their eggs than do the heavy breeds when hens of both types are maintained on the same diet (Scrimshaw *et al.*, 1945; Mayfield *et al.*, 1955; Howes and Hutt, 1956). Since Ellis *et al.* (1933) have shown that hens on diets high in thiamine put more of that vitamin in their eggs than do hens on diets low in thiamine, it seems likely that the amount of thiamine found in the egg is some indication of the excess above requirements of the hen that laid it. Accordingly, among hens on the same diet, those using the least thiamine for their metabolic processes would have the greatest surplus and would deposit the most in their eggs. On this reasoning, the higher thiamine content of White Leghorn eggs would indicate that thiamine is utilized more efficiently by that breed than by the heavy ones.

From crosses made by Lamoreux and Hutt (1939), it seems probable that the difference between White Leghorns and heavy breeds in the utilization of thiamine is polygenic, but we have no idea how such an interesting physiological difference became a part of the breed characteristics of the modern Leghorn. It is thus far inexplicable on the bases of natural selection, artificial selection, or pleiotropic effects of genes that determine the breed. Genetic differences in thiamine requirements within a breed have also been identified, and Light and Cracas (1938) have shown that strains of rats differ in their requirement of thiamine. Presumably similar differences could be developed in the fowl and other species by selection.

Vitamin D. Growing chicks and laying hens need vitamin D for adequate formation of bone and egg shells, but Olsson (1936) found that some White Leghorn hens are able to manage very well without any supplement of vitamin D whatever, while others cannot do so. Such differences within one breed have not yet been shown to have a genetic basis, but there is ample evidence from Olsson's (1948) later experiments that there are significant differences between breeds in the amount of vitamin D required. As in so many other cases, the Leghorns are more fortunate than the heavy breeds, for the latter apparently require nearly three times as much vitamin D when supplied as a supplement in the feed as do the Leghorns. The difference is also found when vitamin D is supplied by irradiation of the skin with ultraviolet light. In this case Olsson found that it takes more than three times as much irradiation to effect normal calcification in Rhode Island Reds as it does in White Leghorns. Similar differences among strains of rats in ability to survive on diets deficient in vitamin D were found by Gowen (1936).

Vitamin E. Broiler growers occasionally have trouble with a type of encephalomalacia that nutritionists ascribe to a deficiency of vitamin E. Even when an ample supply of that vitamin has been mixed in the feed, it is occasionally destroyed by oxidation before the feed is consumed. Chicks with encephalomalacia show ataxia, tremors, retraction of the head, and spasmodic movements of the leg. Sections through the brain show minute hemorrhages and other lesions usually localized in the cerebellum.

This condition can be induced experimentally by feeding a diet high in fat. Using that technique, Howes and Hutt (1952) showed experimentally that White Leghorns are much more resistant to a deficiency of vitamin E than are Rhode Island Reds and Barred Rocks. These findings agree with observations in the field that Leghorns seldom have trouble with encephalomalacia. Carnaghan and Markson (1956) report that 95 per cent of the cases examined by them occurred in heavy breeds. More than a breed difference is involved, however, for Ranby and Outridge (1954) found that the incidence of encephalomalacia in six different sire families varied from 0 to 23 per cent. From the practical standpoint of reducing encephalomalacia in flocks of broilers, it is obviously desirable to provide the current generations with enough vitamin E to protect all the birds, but to practice selection that will ensure that future generations are not handicapped by biologically unfit individuals with abnormally high requirements of that vitamin.

Riboflavin

Among all the genetic variations in the utilization of nutrients none is more interesting than those influencing the use of riboflavin. That vitamin is essential for development of the embryo during incubation and also for growth of the chick after hatching. Ordinarily, hens on diets adequate in riboflavin deposit enough of it in their eggs to ensure normal development of the embryo. Embryos incubating in eggs deficient in riboflavin show a peak of mortality at 10 to 14 days of incubation. As Brown (1957) has shown, when hens on a diet deficient in riboflavin are given a supplement of that vitamin, it takes about 3 weeks to raise the amount of riboflavin in the egg to a maximum and fairly stable level of about 210 μg.

Breed differences. It has not yet been demonstrated that there are breed differences in the requirement of riboflavin for normal development of the embryo. However, Mayfield *et al.* (1955) reported that the riboflavin content of their White Leghorn eggs was 221 μg. against 184 for New Hampshires, the difference being highly significant statistically.

Polygenic variation. Familial differences among White Leghorns in the requirement of riboflavin were noted by Davis *et al.* (1938), who found that on diets deficient in that vitamin the hatchability of fertile eggs ranged in 5 families from 2 to 63 per cent. Further evidence of quantitative genes affecting its utilization was found by Lamoreux and Hutt (1948), who selected for ability of chicks to grow on diets deficient in riboflavin. During 6 generations, 2 distinct strains were differentiated, one comparatively resistant to a deficiency of the vitamin and the other more susceptible than unselected controls. Resistance to the deficiency was demonstrated not only by better survival to 5 weeks of age but also by significantly better growth. As the 2 strains did not differ in these respects when tested on a diet containing ample riboflavin, it was considered that the genetic differences between them were concerned specifically with the utilization of riboflavin.

Riboflavin and melanin. The once-popular sex-linked cross of Barred Rock females × Rhode Island Red males yields female chicks that are solid black and males characterized by a white occipital spot and somewhat grayer down color than in the females. A few years ago hatcherymen reported that among the black female chicks there were always a few with bare backs. As such chicks were unsalable, a study of the problem was requested. The results were most interesting.

To make a long story short, it was found that the hatcherymen's problem was 5 times as great as they had imagined it to be (Hutt, 1951). In other words, for every bare-backed female that they had to discard, 5 more female chicks died during the last week of incubation, mostly in the last few days. Many of these black chicks showed the clubbed down that is characteristic of a deficiency of riboflavin in the diet of the hen, but since the flocks supplying hatcheries are nowadays provided with diets supposedly adequate in riboflavin, it was difficult to believe that the black female embryos could have died from a deficiency of that vitamin. Subsequently, controlled experiments by Bernier and Cooney (1954) proved that on diets somewhat deficient in riboflavin black embryos experienced higher mortality during incubation than their full siblings of other colors. When a supplement of 220 μg. of riboflavin was provided through the air cell to the developing embryo, the rate of mortality decreased, and the black chicks were able to hatch much better than before.

Together, these two studies showed that in black chicks there is some peculiar conflict between the production of melanin and the utilization of riboflavin. In other words, the black chicks have abnormally high requirements of riboflavin. This handicap is lethal to only a few of them. In the cross referred to above, the proportion of black female embryos unable to survive on the level of riboflavin provided by a normal diet was no higher than 4 per cent, but it was consistent during a 10-year period. Even in the chicks that hatched apparently unscathed, evidence of the conflict was found in the fact that black ones had significantly shorter down feathers than their siblings that were not black.

Controlled genetic tests proved that this biochemical battle could be averted by adding to the genotype of the black chick the gene *B*, which causes barring. Even in the heterozygous state, that gene spares the embryo from much of its struggle to form melanin on a limited budget of riboflavin. As a result, from the sex-linked cross described above, the barred male chicks hatch normally, without bare backs, and with longer down than that on their nonbarred black sisters (Hutt, 1951).

TABLE 1

EFFECTS OF THE GENE *RD* ON LEVELS OF RIBOFLAVIN (MICROGRAMS PER GRAM) IN HENS OF THREE GENOTYPES*

Where measured	*Rd Rd*	*Rd rd*	*rd rd*
In blood of hens not laying	0.008	0.008	0.008
In blood of laying hens	0.434	0.272	0.008
In egg yolk	4.30	2.50	0.41

* Data of Buss *et al.* and Boucher *et al.*

Just why it should take more riboflavin to grow embryos with black down than to grow those with other colors is still a mystery, but it should provide an interesting problem for some biochemist.

Blocked transfer. A few years ago a poultry breeder in Pennsylvania reported to an alert representative of a feed company that some of his hens produced fertile eggs, not one of which could be hatched. Study of this problem has provided a fascinating story. Eventually the White Leghorns that could not reproduce found their way to Pennsylvania State University, where studies by Maw (1954) showed that the underlying trouble was caused by a simple recessive, autosomal gene in the homozygous state. Hens with that genotype were unable to transfer riboflavin from the feed to the egg. As a result, none of their embryos survived beyond 14 days of incubation. However, when the incubating eggs of these peculiar hens were given injections of riboflavin before the critical 10 to 12 days of incubation, most of them hatched in the normal manner, and Maw was able to carry on genetic studies with the birds thus artificially produced. Heterozygotes apparently put enough riboflavin in their eggs to permit normal hatchability, but segregation in an F_2 generation and in a backcross showed clearly that homozygous defective birds were produced in the proportions expected for a simple recessive character.

Subsequent studies by Buss *et al.* (1959) and Boucher *et al.* (1959) have shown that the causative gene *rd* is not completely recessive, for heterozygotes have levels of riboflavin in their blood and in the egg yolk that are only a little more than halfway between the levels for the two homozygotes (TABLE 1). They also found that supplements of riboflavin injected into the eggs prolonged the life of the embryos by degrees proportionate to the dosage until that dosage reached 60 μg. per egg. At that level 96 per cent of the embryos in the originally defective eggs were able to hatch normally.

This case provides the only one known thus far in the fowl in which a simple recessive mutation completely prevents utilization of some essential nutrient. All of the other differences mentioned in this report are clearly genetic but are more likely polygenic in nature.

Altogether, the riboflavin story shows that there are: (1) genetic differences between breeds in the utilization of that vitamin; (2) quantitative genes affecting requirements within a breed; (3) a peculiar conflict between formation of melanin and use of riboflavin; and (4) a simple recessive mutation that blocks transfer by the hen from the feed to the egg. It seems probable not only that there may be still other causes of variation in the utilization of riboflavin, but also that equally complex situations may exist with respect to the utilization of most other nutrients. Although this survey has been limited almost exclusively to the fowl, it is altogether likely that similar genetic variations exist in other vertebrates, and it is to be hoped that geneticists, biochemists, physiologists, and others will collaborate in the search for further knowledge about them.

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FAMILIAL HYPOPHOSPHATEMIA: AN INHERITED DEMAND FOR INCREASED VITAMIN D?*

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Much of what is known and some of what is assumed about inorganic phosphorus homeostasis and the syndrome known as "familial hypophosphatemia" are shown schematically in FIGURE 1. The left half of the diagram, designated "normal," shows that the regulation of inorganic phosphorus level is complex. The right half of the diagram attempts to indicate by comparison what the laboratory alterations are in familial hypophosphatemia ("uncomplicated" vitamin D-resistant rickets) and how they come about.

Inorganic phosphorus homeostasis may be described briefly (omitting the qualifications) by noting that phosphorus enters by mouth, is absorbed from the intestine, and then enters the serum pool. The amount of phosphorus in the pool at any time is a result of the balance among the fluxes indicated in FIGURE 1. Phosphorus is continually being deposited and removed from soft tissue and bone. A large part of the serum inorganic phosphorus also is filtered at the glomerulus each minute, most of it being reabsorbed in the tubules. The remainder, a small fraction, is excreted in the urine. Tubular reabsorption, which controls the excreted fraction, is the chief determinant of serum phosphorus level. The amount of reabsorption is regulated reciprocally by the parathyroid hormone. *Increased* parathyroid hormone output *decreases* reabsorption in the tubules (thereby increasing urinary excretion). The decreased reabsorption results in a lowered serum inorganic phosphorus. Parathyroid hormone output is controlled in part at least by the level of serum calcium. A rising calcium level *depresses* the parathyroid, thereby *increasing* tubular reabsorption of phosphate and elevating the level of serum inorganic phosphorus. A drop in serum calcium tends to cause a drop in serum phosphorus by reversing the process described above. A second type of mechanism acting to regulate calcium and phosphorus levels is not shown in FIGURE 1. This is the Ca × P solubility product. Solubility relations probably cause reabsorption of the two substances from bone and soft tissues when the solubility product is below a critical level and deposition of the two from the serum when the solubility product is above a critical level.

The several explanations of familial hypophosphatemia have been reviewed at length in our definitive publication.[1] It is sufficient at this point to note that all the facts are not yet in and that there is still uncertainty about the basic defect. The remainder of this paper describes the insights that we and others have obtained recently by genetic and biochemical approaches.

"Uncomplicated vitamin D-resistant rickets" is the term usually given in the

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literature to the condition we now refer to as "familial hypophosphatemia." It can be differentiated from the other varieties of resistant rickets by differences in the laboratory findings. Unlike the so-called Fanconi syndrome, there is no glycosuria or significant aminoaciduria. In contradistinction to renal tubular acidosis, there is no upset in serum chloride and bicarbonate, and nephrocalcinosis is absent. Familial hypophosphatemia differs from hypophosphatasia, a superficially similar condition, in usually showing a normal or elevated serum alkaline phosphatase.

We encountered a large family with vitamin D-resistant rickets when a 4-year-old boy was referred to our Pediatric Clinic. The boy had the clinical and radiological stigmata of active rickets and hypophosphatemia despite 15,000 I.U. of vitamin D per day (35 times the usual maintenance dose). The details of our experience with him are described elsewhere[1] but may be stated briefly as follows. The patient's bone lesions responded to massive doses of vitamin D, but this therapy led to the laboratory findings of vitamin D intoxication (elevated serum calcium and phosphorus). The calcium level returned to normal when vitamin D was removed from the diet, but the phosphorus returned to its previous low level, and the rickets became active again. It became necessary to titrate him for the dose of vitamin D that would cause healing of the bone lesions but not cause intoxication. The serum calcium and phosphorus and bone X rays were used as determinants.

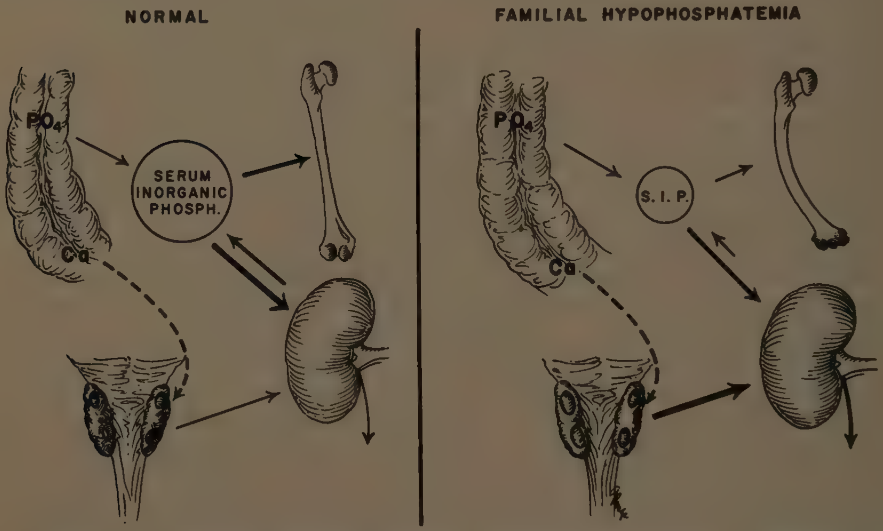

FIGURE 1. A schematic diagram of inorganic phosphorus homeostasis. A difference in the size of the arrows between the two diagrams indicates the type of change and its approximate magnitude. Key: S.I.P. = serum inorganic phosphate.

Examination of the boy's mother showed that she also was hypophosphatemic but that she did not have diagnosable bone disease. A family history that revealed many other affected persons led to an extensive family study that consisted of examination for bone lesions and determinations of fasting serum inorganic phosphorus. It became apparent immediately that a discriminant for serum phosphorus was needed with which to score our subjects as normal or hypophosphatemic. This necessitated study of a normal population and establishment of the normal limits. The details of the study of 511 normal men and 397 normal women of all ages can be found in another place.[2] As a result, serum inorganic phosphorus (Fiske and Subbarow method) was found to be a function of both age and sex. The lower 99 per cent confidence limit for each sex at any age was found to be a good discriminant for defining hypophosphatemia. Any person whose fasting phosphorus level fell below this point was scored as abnormal. A level above this point was used to define a normal person. Clinical studies were also done on many members of the kindred. These included history, physical examination, and bone X rays.

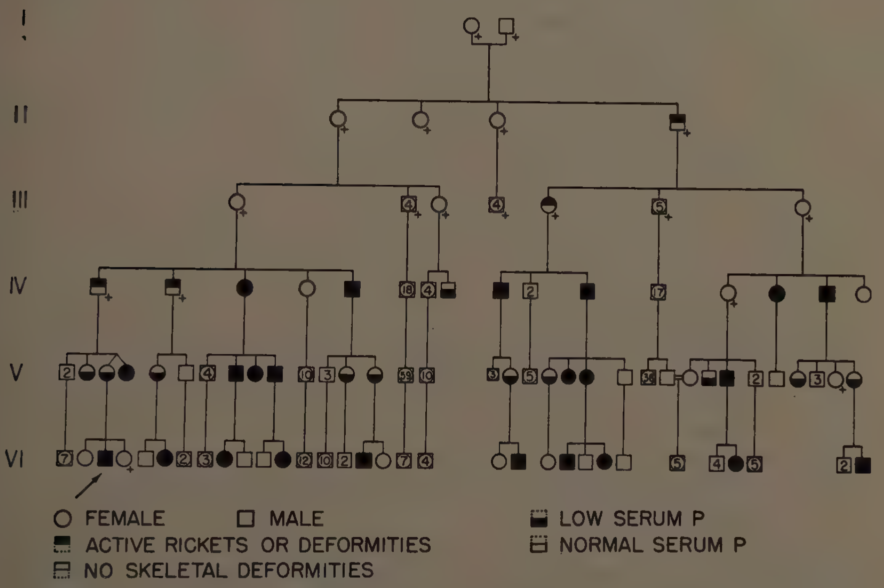

FIGURE 2. The "E" kindred with sex-linked dominant familial hypophosphatemia (vitamin D-resistant rickets).

The first family so studied ("E" kindred) is shown in FIGURE 2. Here the persons studied have been scored for both bone disease and serum inorganic phosphorus levels. It is apparent that some persons had both bone lesions and lowered serum phosphorus. It is also apparent that some persons had lowered serum phosphorus without bone lesions. No person examined had bone lesions *without* reduced serum phosphorus. The pedigree chart also shows that the descendants of some persons with reduced serum phosphorus alone had both bone lesions and reduced serum phosphorus. Taken together these facts suggest that the reduction in serum phosphorus in this family is a more basic expression of the abnormality than the bone lesions.

The environment was examined closely for any agent that might have caused a reduction in serum phosphorus, but none could be found. This fact and the presence of hypophosphatemia in widely separated portions of the kindred covering at least five generations suggested Mendelian inheritance.

We have encountered and studied three other families with this disorder since our original study. The disease is similar in all four families, so the data have been collated for genetic testing. The details of the study of the additional kindreds can be found elsewhere.[3] TABLE 1 shows that the study encompassed a large number of persons (696 total; 526 examined). Our impressions of the disease are based on a group of 78 abnormal persons, which is almost as many as the total number of affected persons cited in the remainder of the literature. This group of 78 hypophosphatemic persons included 40 with either active rickets (children) or osteomalacia (adults).

TABLE 1

FOUR KINDREDS WITH SEX-LINKED DOMINANT FAMILIAL HYPOPHOSPHATEMIA

Kindred	Number of persons	Number studied	Number of hypophosphatemics	Number with bone lesions	
				Examined	Examined + hist.*
E	331	212	37	19	25
B	208	189	20	11	16
H	112	97	13	6	9
L	45	28	8	4	5
Total	696	526	78	40	55

* Adequate history of bone disease in person now dead.

TABLE 2

PROGENY OF HYPOPHOSPHATEMIC PARENTS

Hypophosphatemic parents	Males		Females		Total classified children
	Affected	Normal	Affected	Normal	
22 Mothers	20	13	10	25	68
14 Fathers	0	16	21	0	37
36 Parents	20	29	31	25	105

Ratio affected : normal—Exp. 52.5; Obs. 51:54.
χ^2_T(Exp. 1:1:1:1; Obs. 20:29:31:25) = 2.22; $P > 0.10$.
χ^2_{Mo}(Exp. 1:1:1:1; Obs. 20:13:10:25) = 6.43; $P < 0.02$.
χ^2_{Fa}(Exp. 1:1:1:1; Obs. 0:16:21:0) = 37; $P = (1/2)^{37} = 1.37 \times 10^{-11}$.

In seeking to test the various monogenic Mendelian ratios, we consolidated the progeny of hypophosphatemic parents. These are shown in TABLE 2. There were 22 mothers and 14 fathers with hypophosphatemia. A total of 105 classified children were obtained from these 36 parents. There were 51 with hypophosphatemia and 54 with normal serum phosphorus. This seems to form a 1:1 ratio and suggests a Mendelian dominant trait. In fact, the parents produced 4 classes of children in almost equal numbers: 20 affected males; 29 normal males; 31 affected females; 25 normal females. When the children of affected mothers and fathers are analyzed separately, however, a remarkable result is seen. The mothers produced 4 classes of children, but the ratio departed from 1:1:1:1. We have discussed the meaning of this departure from expectation elsewhere[2,3] and believe it to result from a bias in the discriminant. The hypophosphatemic fathers produced only 2 classes of children, normal sons and hypophosphatemic daughters. The probability that such a finding had occurred by chance with an autosomal dominant gene is in the neighborhood of 1.37×10^{-11}. Such a distribution is characteristic, however, of a sex-linked dominant trait. We have concluded that the abnormal gene responsible in these families for hypophosphatemia is dominant and probably carried on the X chromosome. No mating between two affected persons was found, hence we do not know what the homozygous state is like.

In the pedigree chart (FIGURE 2) it can be seen that the bone lesions sometimes skipped a generation. Tabulation of the "skipped" persons invariably revealed two things: (1) every person skipped was a female; (2) all skipped persons showed hypophosphatemia. Penetrance for bone disease was only 43 per cent, but penetrance for hypophosphatemia was 100 per cent.

The bone lesions are always much more severe in hypophosphatemic males than females. We are not certain how the sex difference in severity of bone lesions is brought about. The only thing we do know is that the level of serum inorganic phosphorus of hypophosphatemic boys is slightly but significantly lower than that of hypophosphatemic girls. The difficulties surrounding the problem of this sex difference have also been discussed at length elsewhere.[1-3] We would probably ignore the sex difference in phosphorus levels (only 0.46 mg. per cent) except for the experimental findings of Fraser and his colleagues,[4,5] who have biopsied costal cartilage in affected children and observed that the cartilage did not calcify in their own serum but that it did calcify in normal serum. These investigators also observed that the addition of phosphate to the abnormal serum resulted in calcification of the cartilage from the genetically abnormal persons. They have also demonstrated that I.V. infusion of phosphate will result in healing of the bone lesions.

Another fundamental question is: Why the hypophosphatemia? To approach the answer we studied the maximal ability of the kidney tubules to reabsorb phosphate. The results are shown in TABLE 3. It was found that the normophosphatemic siblings of affected persons reabsorbed phosphorus normally. The hypophosphatemic persons, however, showed reduced ability of the renal tubules to absorb inorganic phosphorus. This ability was reduced to approximately 50 per cent of normal, a reduction similar to the average 50 per cent reduction in serum phosphorus of the hypophosphatemic persons. It is interesting to note that although the ability to reabsorb phosphorus was reduced more in persons with overt bone disease than persons without overt bone disease, the groups overlapped considerably and were not significantly different.

The cause of the tubular reabsorptive defect is obscure. It is possible that there is a local anatomic or physiologic defect in the tubule itself. It is also possible that the affected person exists in a congenital state of mild hyperparathyroidism. This suggestion has achieved more than armchair status lately because of another of Fraser's experiments.[6] Fraser has shown that when calcium is infused intravenously into affected persons phosphate reabsorption increases in the renal tubules and serum phosphorus rises as though parathyroid hormone output had been depressed.

Still another possible basic cause of the disorder is an absorptive defect in the gut. It is clear that future studies of affected persons should aim at microdissection and histochemical studies of kidneys and intestine, histologic examination of parathyroids, and more extensive and careful calcium-phosphorus balance studies including, perhaps, isotope uptakes.

Finally, it should be pointed out that there are several genetic types of "uncomplicated" vitamin D-resistant rickets. We have recently reported a case not fitting the sex-linked dominant hereditary pattern.[7] If anything has been learned from human biochemical genetics it is that genetic heterogeneity usually implies biochemical heterogeneity. Therefore, interesting biochemical differences might be brought to light by intensive comparative study of the genetically different affected persons.

Table 3
Maximal Tubular Reabsorption of Phosphorus

Subjects	Individual*	Mean*	Standard deviation*
8 Controls	—	137	7.5
4 Hypophosphatemics (with bone lesions)	79 70 65 55	67	10.0
4 Hypophosphatemics (without bone lesions)	85 80 72 66	76	8.4

* (μM of P/100 ml. glomerular filtrate/min.).

It should now be clear that the title of our paper is spurious. Familial hypophosphatemia is clearly *not* an inherited demand for increased vitamin D. Rather it is a condition of upset phosphorus homeostasis. Vitamin D has only an alleviating effect and then only in massive amounts. The vitamin probably acts only to increase the intestinal absorption of calcium and phosphorus.

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GENETIC ERRORS OF METABOLISM AND ENVIRONMENTAL INTERACTION: A SYNTHESIS*

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It seems appropriate to open this article with a comparison of our present knowledge of genetic errors of metabolism with what we knew about mongolism about a decade ago. In a paper published by The New York Academy of Sciences in 1954, Penrose[1] stated that "the susceptibility to mongolism may be partly genetically determined and, if so, then the underlying constitution, though rarely manifested, must be common. This is shown by the high incidence in the general population at late maternal ages where manifestations of the genetical influence may be nearly complete. It is also implied by the low familial concentration of either fully affected or abortive cases. It is most natural, however, to suppose that some infants are more susceptible by virtue of their inherited constitutions than others to an unfavorable maternal environment." As recently as 1959, Ingalls[2] stated that "mongolism, by contrast, does not run in families, nor do mongoloid patients mature and reproduce. Since the condition occurs about once in a thousand births, it defies comprehensive interpretation as a genetic manifestation." The development of techniques for the proper description of chromosomal complement in man has clarified this confusion with regard to the genetic and environmental influences in mongolism. It now appears that in the majority of instances mongolism appears among the offspring of older women through nondisjunction resulting in 47 chromosomes.[3] These are not familial in nature, and the probability of a recurrence in the same family is not great. However, in a small group of families, there appears to be a translocation of chromosome No. 21 to chromosome No. 15, resulting in 46 chromosomes among the mongoloid children and 45 chromosomes in the mothers of these children.[4] This form tends to be familial, the abnormality being transmitted through the female members on the maternal side. Thus there remains little to suggest that environmental factors play a significant role in the occurrence of mongolism, although they undoubtedly influence the expression of some of the secondary manifestations of the condition.

Unfortunately, the interplay between genetic and environmental forces in most situtations cannot be defined in such clear fashion as in mongolism. Instead it is necessary to try to delineate them as well as possible with the limited information presently available. One convenient way of doing this is shown in FIGURE 1. The horizontal axis shows the extent of genetic influences, and the vertical axis shows the role played by environmental influences. Thus a disease like hemophilia or phenylketonuria, which is entirely genetically determined, would fit into position A5. Most workers would agree that diabetes mellitus

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is at least in part genetically determined; yet diet and mode of living may play a role in its pathogenesis, and hence it should probably be placed in position C3. A condition such as epilepsy cannot be considered as a hereditary disease in the classical sense; yet similar abnormal electroencephalogram tracings are noted with great frequency among families, especially among the twins of affected individuals. This would probably place epilepsy in position D2. Finally, chicken pox and lead poisoning, although they may run in families, are almost certainly due to only environmental factors, and these diseases should be placed in position E1.

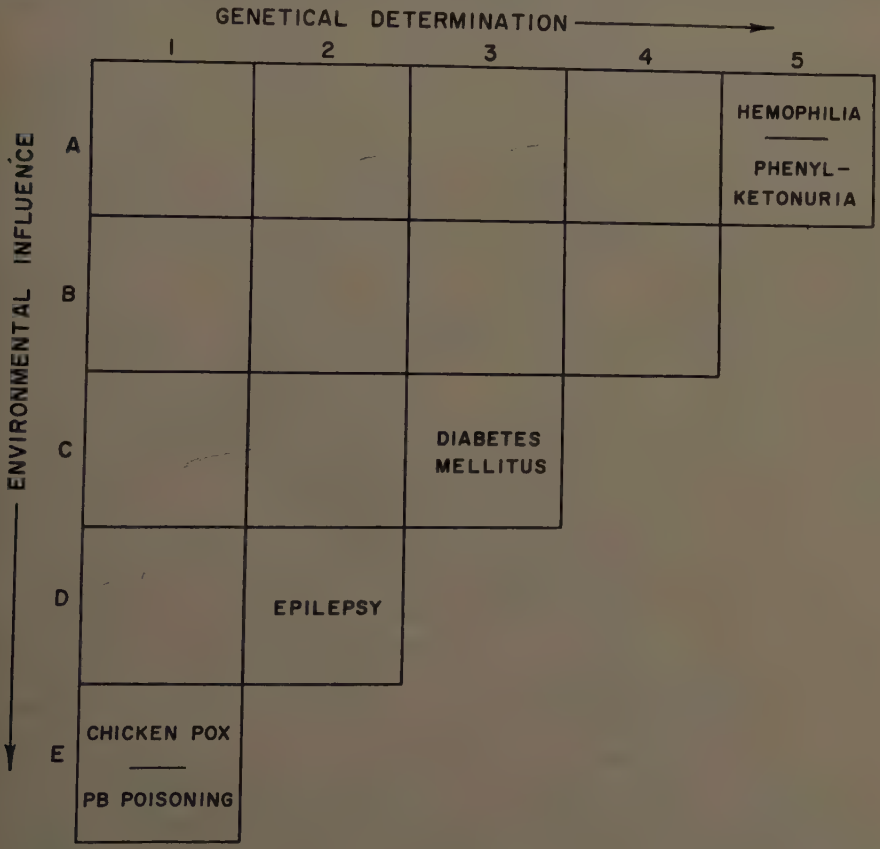

FIGURE 1. Method for delineating hereditary and environmental factors in the pathogenesis of disease. Reproduced by permission of The Year Book Publishers, Inc.[28]

With these factors in mind, I suggest that both genetic and environmental forces are at play from the moment of conception or, possibly, even prior to that, all the way through adult life. For the sake of convenience, this will be discussed chronologically under the following headings: (1) prior to fertilization; (2) during embryogenesis, (3) during the immediate prenatal and postnatal period; and (4) after early infancy.

Even prior to fertilization, gross abnormalities of the chromosomal constitution will result in such sexual aberrations as the Klinefelter's syndrome and Turner's syndrome. It will also result in such severe malformations as poly dys-spondylism and mongolism. Presumably the more severe disturbances of chromosomal constitution are lethal, and either the fertilized egg fails to develop or it is aborted at an early stage of gestation; there is accordingly no opportunity to make a proper study of these defects. Recent studies have shown that even relatively small doses of radiation can cause serious chromosomal changes in man.[5] It is more than likely that teratogenic agents can also effect such changes, which account for the fact that approximately 1 of every 10 pregnancies end in this condition. It also appears that this is a rich area for investigation as a result of the new chromosomal techniques.

Now for a consideration of the genetic and environmental influences that are likely to alter early embryogenesis: at this point, there is ample evidence to indicate that congenital anomalies can be experimentally produced by means of external factors such as anoxia,[6] deficiencies of certain vitamins and metabolites,[7] and the injection of cortisone in pregnant females.[8] One example may be cited: folic acid deficiency induced by omission of folic acid in conjunction with the administration of an antimetabolite (X-methyl folic acid) during the second half of gestation in the rat results in various degrees of openness of cleft palate.[9] If the deficiency is sustained for the 9th to 11th, the 10th to 13th, or the 8th to 10th days of gestation only, added facial deformities are obtained, such as oblique facial clefts, harelip, and "open" eyes. Furthermore, complex differences in the appearance of the palate become manifest. In the long-term deficiency, failure of adequate breadth and depth of mandibular development may result in inability of the tongue to regress; this, in turn, may be a simple mechanical explanation for failure of the palate to close. In short-term, acute deficiencies the cleft palate may be asymmetrical. The median nasal process grows down and "attempts" to make a palate; the spread results in the formation of a bilateral cleft palate.

There is equally good evidence that congenital anomalies can result from purely genetic factors. Lorincz elsewhere in this monograph describes a disturbance of mucopolysaccharide metabolism in patients with heritable multiple exostoses. This disturbance is clearly an "inborn error of metabolism" that occurs *in utero* and results in structural and morphological changes after the fetus is born. It is more than likely that the majority of the congenital anomalies regarded as genetically determined represent blocks in normal metabolism during various phases of embryogenesis and differentiation. In many instances the major change has already occurred by the time of birth, and it is no longer possible to detect the abnormality biochemically.

A much more complex subject is the interplay between genetic and environmental influences during this critical stage in development; of this little is known. I can only give Wilson's[10] schematic representation (FIGURE 2) of the relationships between the growing embryo and its environment and quote Paul Weiss[11] in a recent symposium "Environmental Influences on Prenatal Development":

"If we view development as a whole, we cannot accept a sharp dichotomy of geneticists versus environmentalists. Development is the result of a long process of continuous changes, starting from a given genetic set-up, interacting with a given environment. The first interaction product constitutes the background for the next step of interaction with the environment, which in the case of a given cell includes the interaction product of other cells, and in this manner all future reactions are co-determined by all preceding steps and hence by genetic endowment. Anywhere along this line, interventions may unbalance the system. There are presumably general metabolic requirements held in common by all cells, whose derangement by certain agents will produce sweeping effects. But, also different tissues progressively develop very different metabolic needs, and therefore certain other agents will affect only specific partial processes and only at times when such need may be critically interfered with. As a result, we are faced with a wide spectrum of interference and defects from very general to localized ones."

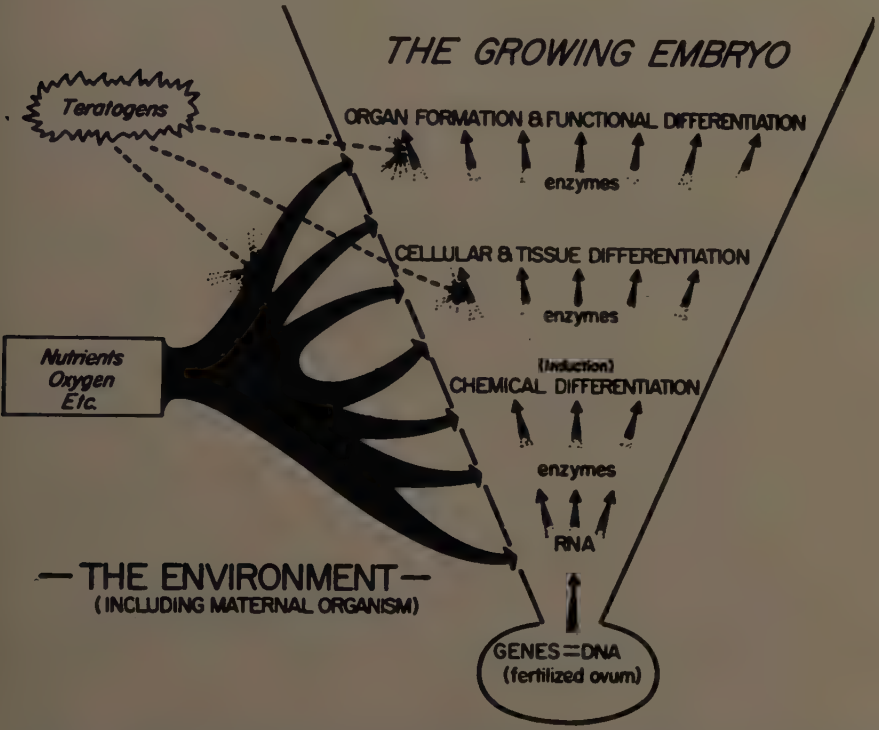

FIGURE 2. Scheme illustrating the dependence upon environment of the mammalian embryo at all levels of development for the materials needed in growth. Reproduced by permission of the *Journal of Chronic Diseases*, The C. V. Mosby Co.[10]

I now turn to a consideration of the metabolic factors that are likely to play a role in the prenatal and immediate postnatal period. There is currently much information available on the various metabolic changes that take place during early ontogenesis; generally, functional differentiation coincides with major enzymatic developments.[12] The smooth functioning of enzyme systems in the developing fetus can be upset by various mechanisms, as shown in the following examples:

(1) There may exist a genetically-determined deficiency of one of these enzyme systems resulting in an "inborn error of metabolism." In galactosemia, infants tolerate milk poorly, become lethargic, and fail to gain weight; ultimately death occurs from malnutrition and wasting. This condition, transmitted by an autosomal recessive gene, is caused by a congenital deficiency of the enzyme galactose-1-phosphate uridyl transferase.[13]

$$\text{Gal-1-P} + \text{UDPG} \rightleftharpoons \text{G-1-P} + \text{UDP Gal}$$

Careful studies have shown that the galactosemic infant is affected prior to delivery. Infants with this condition fail to show galactose-1-phosphate uridyl transferase levels in the cord blood, whereas this enzyme is present in normal amounts at birth in unaffected infants. Furthermore, Komrower and his coworkers[14] have shown that there is a several-fold increase of galactose-1-phosphate in the cord blood of affected infants even prior to the ingestion of milk. This would imply that the "error" in metabolism was present prior to birth and that the infant might have been handicapped because of it. Another such example may be found in cystic fibrosis, a generalized disease of exocrine glands, which manifests itself clinically by loss or diminution of pancreatic exocrine secretions, by increased susceptibility to upper respiratory infections, and by elevation of chloride concentrations in sweat gland secretions. In a study involving 123 children with cystic fibrosis and 144 unaffected sibs, it was found that the affected children weighed an average of 250 gm. less than their unaffected sibs,[15] again suggesting a genetic influence even before the baby is born.

(2) During the fetal and newborn period, there exists in many instances a transient decrease of specific enzyme systems, and they rise to normal adult levels only after a period of days or weeks. These can sometimes be profoundly affected by external influences as well as by environmental factors within the mother during pregnancy. This can be illustrated by considering the glucuronosyl transferase system, which is responsible for the conjugation of bilirubin:

$$\text{Bilirubin} + \text{UDPGA} \rightleftharpoons \text{bilirubin glucuronide} + \text{UDP}$$

Physiological hyperbilirubinemia in the newborn infant is believed to result in part from a transient deficiency of this enzyme system during early infancy, as shown in FIGURE 3.[16] Lathe and Walker[17] have shown that the sera from pregnant women will inhibit this enzyme system *in vitro*. These findings have been confirmed in my laboratory, as shown in FIGURE 4; the inhibitor has been identified as pregnanediol.[18] Thus we have *in vitro* evidence, at least, that certain physiological changes in the pregnant woman might play a role in influencing this enzyme system. The fact that the young fetus already has a deficiency of this enzyme may make this doubly important.

(3) This example concerns development after birth. It is well known that the excessive levels of phenylalanine in phenylketonuria cause an inhibition of 5-hydroxytryptophan decarboxylase, and this in turn results in a decrease in the levels of 5-hydroxytryptamine in the blood and of 5-hydroxyindoleacetic

acid in the urine of such patients.[19] It is also known that the low phenylalanine diet is effective in preventing mental deficiency in such children only if started at a very young age.[20] During the past year, we have found a relative deficiency of 5-hydroxytryptophan decarboxylase in the rat kidney homogenate during early infancy (TABLE 1).[21] This would suggest that in phenylketonuria this relatively inadequate enzyme system is further inhibited by an excess of phenylalanine derivatives, which may result in even further lowering of the levels of 5-hydroxytryptamine, a critical component of the brain. This may in part explain the relative effectiveness of lowering the phenylalanine level early in infancy and the failure of older phenylketonuric children to show the same response.

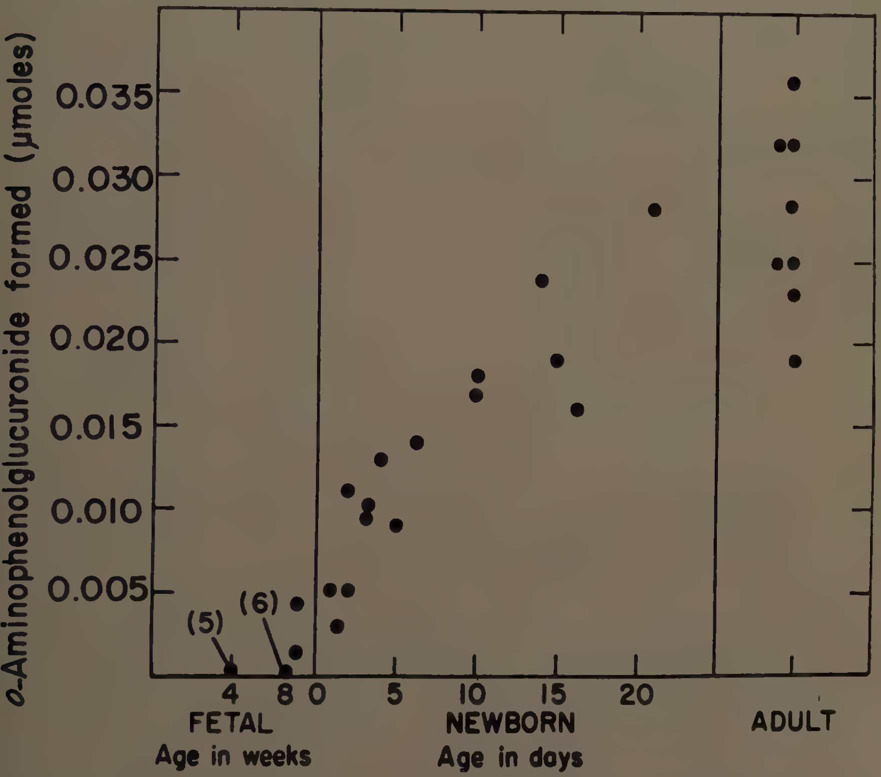

FIGURE 3. Comparison of the amount of *o*-aminophenolglucuronide formed by fetal, new born, and adult guinea pig liver. Reproduced by permission of the *Journal of Clinical Investigation*.[16]

Finally, we consider the environmental influences upon hereditary metabolic defects in the individual after the time of birth. A few examples follow:

(1) A survey in Maryland of children with adrenal hyperplasia revealed an excessive number of females.[22] This may be attributed to the fact that the androgenic effects in the female result in pseudohermaphroditism, which is easily diagnosed shortly after birth, when prompt therapy can be instituted. In the

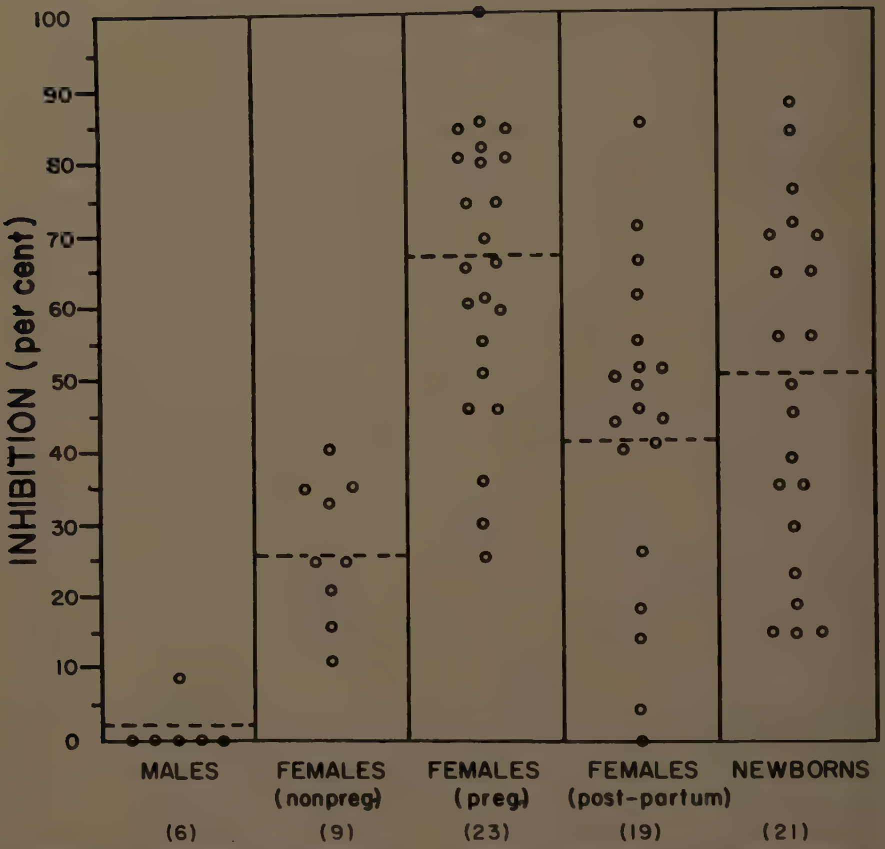

FIGURE 4. Inhibition of *o*-aminophenol conjugation by various types of sera. Incubation mixture consisted of: 0.5 M Tris buffer, pH 8.0, 0.1 ml.; 0.5 M magnesium chloride, 0.1 ml.; 1.1 × 10^{-3} M uridine diphosphoglucuronic acid, 0.1 ml.; 1.25 × 10^{-3} M *o*-aminophenol in 0.01 M ascorbic acid, 0.2 ml.; 10 per cent rat liver homogenate, 1.0 ml.; and serum, 0.22 ml. Incubation was carried out at 37° C. for 30 min. Reproduced by permission of *Nature*.[18]

TABLE 1

KIDNEY 5-HYDROXYTRYPTOPHAN DECARBOXYLASE ACTIVITY IN RATS OF DIFFERENT AGES*

Age	No. of exp.	No. of animals	5-Hydroxytryptophan decarboxylase activity†
Fetal‡	3	45	0
1 day	3	23	0
2–5 days	4	24	0.21 ± 0.21
7–12 days	5	26	0.38 ± 0.10
21–23 days	7	21	0.66 ± 0.13
33 days	4	8	1.19 ± 0.47
Adults	14	14	1.34 ± 0.58

* Reproduced by permission of *Nature*.[21]

† Mean values expressed as μmoles 5-hydroxytryptamine formed per 100 mg. of dry weight of kidneys per hour.

‡ One to 3 days before term.

male the macrogenitosomia precox may not appear until a later age; many may die from salt loss and dehydration without the diagnosis having been properly established. Thus selection is at work, often without observers being very much aware of it.

(2) In congenital agammaglobulinemia[23] a number of male relatives on the mother's side have died from overwhelming infections without the etiology being known. With awareness of the use of gamma globulin in the treatment of the affected individuals, the diagnosis is less likely to be missed in the future.

(3) A condition that baffles us frequently is nephrogenic diabetes insipidus. Most of the patients are admitted to the hospital as infants that fail to thrive and become dehydrated relatively easily. Careful investigation, however, fails to reveal a specific cause for this dehydration; a failure of the urine to concentrate frequently provides the only clue as to the pathology in the condition. Unless the definitive concentration test is done both with and without Pitressin, it is difficult to account for the vague symptomatology, and I am certain that the diagnosis in the majority of such infants is missed even today.

The effects of diet upon the development of hereditary diseases are well known. It is generally agreed that excessive carbohydrate intake and obesity are more likely to bring on diabetes mellitus when the individual has the genetic constitution for that condition. Similarly, the ingestion of excessive amounts of meat and foods high in purine content will bring about an attack of gout in a hyperuricemic individual. Diets high in fat will probably influence the incidence of coronary heart disease, at least among individuals with idiopathic hyperlipemia if not in other individuals with a long familial history of hypertension and cardiovascular disease.

Hutt elsewhere in these pages very clearly outlines the genetic variations in the utilization of various vitamins and other nutrients. In this he has a tremendous advantage over investigators dealing with human material: he can influence to a great extent the genetic constitution of his experimental subjects. Unfortunately, except for certain isolates, man is too heterogeneous to permit such definitive differences. However, there is much to be learned from these experimental methods and approaches.

In contrast, many researchers must depend upon materials that have been selected by mutations. Graham and Winters elsewhere in this monograph present an excellent example of an inherited demand for vitamin D that appears to be due to hypophosphatemia, possibly on a renal basis. While there is no disagreement with Graham and Winters' concepts, I should like to extend them to a broader consideration of the needs for vitamin D in man. A whole spectrum of needs for vitamin D might be postulated as shown in TABLE 2. In the hypophosphatemia described by Graham and Winters there is probably a genetically determined disturbance of the renal transport mechanism for phosphorus that results in tremendous requirements; thus there may be signs of vitamin D intoxication while the patient still shows clinical signs of rickets. The usual form of rickets associated with inadequate intake of vitamin D is next observed. This group is particularly intriguing because children in some families develop florid rickets whereas others remain perfectly normal on the same vitamin D intake. Jonxis[24] has suggested that vitamin D-deficient rickets also tends to run in families and may represent a milder disturbance in the renal transport mechanism for the reabsorption of phosphorus. In the center column of TABLE 2 is given the amount of vitamin D needed by the normal individual, who requires a daily intake of about 800 I.U.; as long as he gets that amount an equilibrium is maintained.

About 15 years ago, a number of reports appeared from England describing infants who failed to thrive, who were found to have elevations of their serum calcium levels. Careful investigations of these cases showed that both the milk and cereals provided by the National Health Service were overfortified with vitamin D so that these infants were receiving between 1500 and 2500 I.U. daily instead of the recommended 400 to 800 I.U.[25] The excess vitamin D was removed, and this syndrome is now almost extinct. The important question to raise here is: Why did 200 to 300 babies show mild signs of vitamin D intoxication when millions of others fed on the same diet were free from such signs? It would appear that the level of intake, which is perfectly satisfactory for most infants, was too much for specific babies, suggesting that they have some constitutional or genetic factor that makes them more susceptible to vitamin D. Finally, Fanconi and his co-workers[26] have described a small group of children with severe hypercalcemia characterized by excessive bone density, renal involvement, and mental deficiency. These tend to be more familial in nature. Fellers and his co-workers[27] have shown that rachitic rats can be cured by using only one-fiftieth the amount of serum from such patients as compared with normal people, indicating that their need for vitamin D is considerably less than that of the normal individual and that perhaps they are suffering from vitamin D intoxication even without any intake. Thus we have a whole spectrum of needs for vitamin D: in part genetically determined and in part influenced by environment.

TABLE 2
INCREASED NEED FOR VITAMIN D*

Severe hypocalcemia	Mild hypercalcemia	Normal needs	Deficient rickets	Resistant rickets
0(?)	200(?)	800	5000	100,000

* Given in international units.

To summarize: an attempt has been made to evaluate the genetic and environmental influences that interact prior to conception and throughout adult life. It should be emphasized, however, that in all these situations, the genetic factor is dominant and cannot basically be altered. Although the environmental factors may at times be very prominent and might mean life or death to the individual, ultimately it is the genetic factor that precipitates the situation. There is still much to be learned about the role of both these factors in the developing organism. However, sufficient progress has been made to warrant some practicable speculation on the meaning of these data in terms of future research endeavors.

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DISCUSSION: PART II

NEWMAN: Since Lorincz mentioned bulldogs, Boston terriers, and the like, I ask whether he would get the same syndrome in them as in snorter cattle and in humans. Would this be a feasible research design? With what would he compare the Bostons or the bulldogs?

LORINCZ: The type of dwarfism observed in the bulldog is believed to be a form of achondroplasia, a heritable abnormality of cartilaginous development. In so far as I know, definitive studies have not been made of the urinary or tissue acid mucopolysaccharides in either the achondroplastic human or in such affected animals as the bulldog, beagle, or dachshund.

We have tested grossly (by means of the acid albumin turbidity screening technique) the urine from two or three adult achondroplastic dwarfs; these have been negative. I suggest that we form no conclusions from such meager data. Achondroplasia appears in many different animal species, including man, fowl, cattle, and others. It is quite distinct from the type of dwarfism known as the Hurler syndrome in man, or the type of dwarfism present in the snorter dwarf cattle.

QUESTION: Heparin is well known as an anticlotting agent, and I understand that some of the other chondroitin sulfate derivatives also have anticlotting properties. I ask whether the blood clots perhaps more slowly in these animals and whether, if heparin were injected, it would act quantitatively in the same way as in a normal animal or person.

LORINCZ: It is true that some of the sulfated polysaccharides have antithrombic activity. Chondroitin sulfuric acid-B, that occurs in large amounts in the Hurler syndrome and apparently in the snorter dwarf cattle, does have an *in vitro* antithrombic activity, and this effect seems to vary with the concentration of thrombin present.[1]

To my knowledge, no defects of the clotting mechanism or bleeding tendency have been reported to occur in the Hurler syndrome, or have been noted in the snorter dwarf cattle. Likewise I am unaware of any studies in which the administration of heparin in doses large enough to affect coagulation have been performed on any of these subjects. Whether an altered reaction to such heparin administration would occur apparently remains to be determined.

HARRY SOBOTKA (*The Mount Sinai Hospital, New York, N.Y.*): The Hurler syndrome formerly was considered to be a lipoidosis. During the last 10 years it has been recognized as a storage disease of mucopolysaccharides, and there is a tendency to blame the accumulation of the lipids in various tissues and organs in this disease on secondary mechanical blocking effects. However, there may be a relationship between the storage of lipids in Hurler's disease and the effect of heparin on the so-called clearing reaction in the lipid metabolism of the serum.

You have undoubtedly seen the observations of Karl Meyer on tissue cultures from chondromata and similar tumors where it is claimed that individual cells produced either chondroitin sulfate A, B, or C. I ask whether such considerations could advance the elucidation of the etiology. An enzyme has been described in the skin of rabbits that converts chondroitin sulfate A (CSA-A) into chondroitin sulfate B (CSA-B) by inversion of the carbon atom "5" resulting in the L-iduronic acid configuration.

LORINCZ: The basic biochemical defect in the Hurler syndrome is not yet known. This condition was considered a lipoidosis and, in fact, is still listed as "lipochondrodystrophy" in the *Index Medicus*. Lipids have been implicated, I believe, not so much from the finding of abnormal qualitative or quantitative differences in tissue lipids, but rather on the basis of finding vacuoles present in the parenchymal cells that were presumed to be lipids that had been removed by alcohol fixation of the tissue sections. Subsequent histochemical studies, and later Brante's definitive identification of this material as acid mucopolysaccharide in composition, have altered the concept of this disorder as a simple abnormality of lipid metabolism.

Sobotka's point, about the effect of an increased level of circulating polyelectrolytes able to bring about lipid clearing of serum and perhaps altered lipid deposition in tissues, is an excellent one.

In collaboration with Saul Baker,* we have studied lipid clearing in a preliminary fashion in one child with the Hurler syndrome. This patient had a markedly elevated level of intrinsic clearing factor in the serum as tested by Baker's *in vitro* assay. The level of lipid clearing was capable of being increased further by the injection of a small dose of regular heparin, a dosage capable of eliciting a clearing response in normal serum without affecting coagulation time.

The tumor and tissue culture studies of acid mucopolysaccharide formation are most interesting and may well contribute to our understanding of the metabolism of these compounds in normal and abnormal conditions in animals including the dwarf cattle reported here. I am not acquainted with the enzyme of rabbit skin that converts CSA-A into CSA-B. I should like to learn more about it, since this would be a most significant discovery.

I can say only that the Hurler syndrome need not necessarily represent an enzymatic defect of acid mucopolysaccharide formation or utilization. It is entirely conceivable that there may be an enzymatic defect that primarily affects the linkage of the polysaccharide to the protein component with which it is found normally in tissues, or that may represent some entirely different defect that exerts its effect on acid mucopolysaccharide metabolism only in a secondary manner. In brief, the basic defect in the Hurler syndrome is still not understood.

WALLACE: I ask, with regard to the Australorp, which I presume produced black chickens, why it is so much better than other breeds; also, how much of a job is it to test eggs not merely for riboflavin but for the various other vitamins; and further, whether it would be a good idea to use tests of eggs as an indication of the desirability of breeding from a particular hen.

HUTT: Black Australorps are not pure black at hatching. They have some white feathers, often in the wing tips. Also, the abdomen is sometimes white and, actually, their melanin load is not as heavy as in some of the other breeds.

Analyses of eggs for vitamin content is an unpleasant business, except in the case described by Maw *et al.* (cited elsewhere in this monograph), who spot riboflavin content by a difference in color of the albumen; but to make quantitative tests for thiamine is very difficult.

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Howes, who worked with us, tried bioassays by measuring the rate of growth of a fungus with supplements of the egg and, finally, came to the thiochrome blue method, which I think is standard.

I do not know how difficult it would be to measure the riboflavin quantitatively by a chemical method. I have not done it. I think the reason we have not had more of these tests is that the quantitative assay of some of these vitamins in eggs is not an easy matter.

JOHN W. GOWEN (*Iowa State University, Ames, Iowa*): Hutt has reviewed some exciting areas of gene actions as they control both normal and pathological conditions, particularly chickens. His review of the analysis of Maw *et al.* for riboflavin control by a single gene pair is really significant. The presence of one allele gives life with normal development. The presence of the other results in death. The unavoidable importance of the organism's genotype to disease is indicative of what is done by many other gene pairs making up chicken inheritance. A comparable mammalian case, but one acting through different paths, has recently been studied in our laboratory (Hollander and Gowen[2]).

Some years ago we discovered a hairloss (hl) mutation. This mutation is recessive and is located on the sixth chromosome of the mouse. When it is homozygous recessive (hl/hl), the mouse during nursing grows a normal coat of fur which it retains for from 2 to 3 months; the hair is then shed and the mouse becomes naked. These animals have bare skins and, in the light that humans are similarly defective, show a distinct difference in hair metabolism from the normal wild-type mouse. The hairloss condition becomes the phenotypic mark of this gene, but the gene has other actions that are displayed by the progeny.

Matings (males phenotypically hairloss, hl/hl, bred to normal haired females heterozygous for hairloss, hl/+) would be expected to give 50 per cent progeny hairloss, hl/hl, and 50 per cent full furred, hl/+, as they do. Similarly when the heterozygous males, hl/+, are mated to homozygous hairloss females, hl/hl, the expectation also would be 50 per cent hairloss progeny to 50 per cent fully furred, but these percentages are not realized. Approximately one half of the fully-furred progeny, hl/+, die. This maternal-fetal incompatibility does not show erthyroblastosis. The conditioning factor is the recessive hairloss gene. Normal-furred progeny of hairloss mothers, born alive, show high mortality during the first two weeks of life, even when fostered by normal mothers. The syndrome is marked by inadequate lung inflation, slow growth, and bones that show breaks and swellings around the breaks and joints. Mothers that are nursing previous litters during their pregnancies have progenies particularly prone to these symptoms and almost complete mortality of the hl/+ young. This mammalian case is notable, for apparently it represents a direct determental interaction between what a priori would be considered the most highly perfected allele in the genotype, the wild-type allele, and the mutant hl/hl gene. The products of this interaction act during pregnancy and ultimately lead to a series of pathological conditions, deformities, and deaths, during the nursing period.

COMMENT FROM THE FLOOR: I shall comment on chickens, particularly in relation to this riboflavin problem. I gather from Hutt's observations that there is no information about the form of riboflavin that occurs in the blood of these chicks or the form that occurs in the egg. As you know, riboflavin has difficulty in crossing the human placenta; this situation with the chicken may be something similar. There may be the same problem across the chicken membrane, as far as getting into the egg is concerned.

I suggest that this is an interesting experimental situation to be studied.

HUTT: The different forms of riboflavin involved have been studied by workers at Pennsylvania State University, University Park, Pa. Their deficiency in eggs is entirely in what they call free riboflavin. Deficient birds have, in the eggs and blood, a normal content of flavin adenine dinucleotide, and flavin mononucleotide. These fractions are not affected. It is the free riboflavin that is affected.

GRAHAM: I ask Hutt a question about chickens. We physicians are under attack for acting dysgenically by keeping people with deleterious genes alive and allowing them to reproduce.

My question involves the other end of this spectrum of selection and interference with natural selection: what would happen if the chickens that have been highly selected for various economically useful traits suddenly had to fend for themselves—could they?

HUTT: Some could and some could not. The most unlikely to succeed, in the whole poultry yard at the present time, is the turkey. These big, broad-breasted birds have been developed within the past 15 to 20 years. They are a great improvement over the old, sharpbreasted ones we used to have. There is more meat on them; they look better, carve better, and hotels like them; indeed they are a great addition to the nation's larder. However, they hardly can reproduce themselves; rather, they cannot reproduce themselves. Fertility is so poor in natural matings that turkey breeders must rely on artificial insemination.

If we turned loose these domesticated turkeys in the Pennsylvania woods where their ancestors are subsisting fairly well, or in some other parts of the United States, these domesticated fowls would have no chance of survival.

With respect to some of the others, the situation might be different. I already have praised the White Leghorns. I think that, in some parts of this country, if these birds went feral, they would survive fairly well. I believe eventually their color might disappear and the browns would take over, as they have in other species, but some of the whites will survive. I am uncertain about the heavier breeds; we are not likely to experiment with them on an extensive scale.

C. C. LI (*Graduate School of Public Health*, *University of Pittsburgh*, *Pittsburgh*, *Pa.*): With regard to retaining bad genes in the human population, I recall a story: somebody asked a fashion designer in New York, N.Y., "Can you tell us the trend of women's fashions?" She replied, "I face the same problem as the poultry breeders: I do not know what the customers want—all breast or all legs!"

Whenever we discuss this question of the genetic future of the human population, too often it is taken for granted that we know what we shall want in generations to come. Therefore we are entitled to use the words bad and good;

but like the dress designer, I am confused. What is it that we shall want in the future?

OSBORNE: One should not give the impression that the objectives or purposes of animal breeding and human genetics are the same. Certainly in one area we learn from the other, and because man is not an experimental organism we turn, very profitably, to genetic work with different forms of animals. What are the objectives of the animal breeder? They are to learn about different genetic mechanisms, to discover the types of traits he can breed for, and to breed for such traits as will furnish economic advantage in specific environmental situations.

Our objectives in man, of course, are also to learn about genetic mechanisms, but only for the purpose of understanding these mechanisms in a way that environmental factors that trigger pathological reactions can be identified and controlled, that is, as in the diabetic. In man we are looking for ways to manipulate his environment, not to manipulate the genetic structure of the human population.

In man, most of the genetic traits with which we work, at least as they are expressed on a recognizable level, are due to genetic factors that have pleiotropic effects. These factors when homozygous may give ill effects as, we assume, is the case in diabetes. We do not know what the total effects of these factors in heterozygotes are or what would happen if we were to remove these genetic factors from our population. We might be removing a valuable part of our gene pool. We cannot speculate as to the consequences of the medical saving in the population of some individuals who suffer from serious diseases. Li stated the problem nicely with his anecdote. We do not know where man is going, or what his genetic needs will be in the future.

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Part III. Genetic Resistance and Susceptibility to Infectious Disease

EXPERIMENTAL ANALYSIS OF GENETIC DETERMINANTS IN RESISTANCE TO INFECTIOUS DISEASE*

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Problems of infectious disease susceptibility and resistance have been extensively studied by the epidemiologist, the bacteriologist, and the geneticist. The objectives of these scientists, although expressed differently, have much in common. The epidemiologist, chiefly through the study of spontaneous epidemics, seeks to specify the factors that affect the initiation, the spread, and the regression of the disease. The bacteriologist's considerations are (1) to assign a pathogen as the specific causative agent for the disease and (2) through treatments of this agent to generate means of controlling the disease. The geneticist utilizes both of these approaches as fundamental to his problem but, in addition, directs his attention to the questions of why there should be variations in survival of the hosts that may be exposed to the disease and why comparable variations are observed in pathogen virulence.

The plant breeder through the centuries has recognized that some plants of a species were resistant whereas others were susceptible to the same disease. The culmination of this thinking came when Biffen (1905) interpreted the observed resistances and susceptibilities of certain wheat varieties to yellow rust as due to the action of a single gene pair. This observation has been extended to cover a large number of species of plants and their diseases. Resistance to given diseases within a plant species has been shown to be genetically independent or linked with other characteristics. A locus may be occupied by several alleles, whose phenotypes show graded differences in resistance.

Resistance, as displayed by the different genetic phenotypes, is specific for particular genetic phenotypes in the pathogen. Physiological forms of the rust organisms in cereals are numerous. A single form of rust may invade a host or be resisted by it according to the presence or absence of a single gene in the host inheritance complex. Two or more different rusts may sometimes be controlled by the presence or absence of a single gene. For other rust genotypes, two or more host loci and their proper genes are necessary for similar control. Pandemics of cereal diseases have been rendered less detrimental by world-wide search for host genes that have specific resistances to different disease types.

This hand-and-glove relationship of the host genotypes to the pathogen genotypes is to the liking of the geneticist but has unfortunately seldom appeared in animal material. The all-or-none susceptibility of a given strain or species is seldom approached where the observations are on adequate numbers of individuals. The liver disease of mice intimately associated with the pathogen *Bacillus piliformis* is perhaps as near a case in point. Tyzzer (1917) observed species

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susceptibility differences between *Mus bacterianus* and *Mus musculus*. In an epidemic, 174 cases leading to the death of the mice were observed in *M. bacterianus*; only 2 were detected in *M. musculus*. Scattering cases occurred to the F_5 generation. Follow-up studies on this problem by Gowen and Schott (1933*a*) clarified the disease structure somewhat since provision was made that each mouse be fed a fixed amount of diseased liver. The 37 *M. musculus* showed no deaths. These mice were of 2 inbred strains. Eighty of 99 *M. bacterianus* having the waltzing gene died. F_1 crosses with the 2 *musculus* strains behaved differently. One cross gave 23 per cent susceptible, the other none. Backcrosses to *M. bacterianus* had 56 per cent susceptible mice in the progeny. These results could be interpreted as due to a single gene. However, other data on *M. bacterianus* direct from China, but not carrying the waltzing gene, displayed less-clear species differences. Of 84 Chinese mice tested, 27 per cent died. F_1 crosses to *M. musculus* were completely resistant. F_1 crosses within the *M. bacterianus* species, waltzers × Chinese, had only 14 per cent of the mice die. The backcrosses to the Chinese were, as expected, only partially susceptible but at too low a per cent (25 per cent deaths). These results showed that the Chinese *M. bacterianus* mice were not uniformly susceptible to this disease. As the Chinese mice were random bred, it was not surprising that they showed greater resistance variations than observed for the inbred waltzing mice. While a single major gene difference for the disease susceptibility and resistance syndrome seemed reasonable, it was not proven.

Another case, studied by Rich (1923), is particularly significant. Rich discovered guinea pigs that were deficient in a portion of their complement, the third part. These pigs lacked complement reactions. A single gene difference was found responsible. Inoculated with *Salmonella cholerae suis*, 77 per cent of the no-complement pigs died, whereas only 20 per cent of the pigs having full complement died. The severity of the disease was distinctly influenced by the gene for no complement. The disease reaction did not give the all-or-none phenotypic effects it did for the complement pigs. Instead, disease expression was intermediate, indicating that the background inheritance had an important part in the reaction.

Distribution of Host Resistance

The more common situation is the distribution of host resistance into many different phenotypic classes, each of which has particular attributes: human tuberculosis: Hirsch, 1883; Pearson, 1912; Goring, 1909; Ferguson, 1928; Puffer, 1944; experimental tuberculosis in animals: Wright and Lewis, 1921; Lurie, 1941; Lynch, 1960. These provide illustrations of the varied phenotypes found in one disease. Comparable illustrations are well known for other infections as well as metabolic diseases and those of unknown etiology such as cancer. Possibly the best illustrations come from the work on *Salmonella* diseases of three different host species: *S. enteritidis* in rats, Irwin, 1929; *S. gallinarum* in the domestic fowl, Lambert, 1932, 1935; Lambert and Knox, 1932; Gowen, 1937 *et seq.*, 1954; *S. pullorum*, Roberts and Card, 1926 *et seq.*; *S. typhimurium*, Schott, 1932; Hetzer, 1937; Gowen and students, 1933 *et seq.*, 1960; and *S. enteritidis*, Webster, 1933, 1937. Most of the individual workers have been satisfied to consider only one or two lines derived from random-bred stock.

By progressive selection for resistance or susceptibility they were able to show that the original populations were composed of different breeding types marked by differences in susceptibility to the disease with which they were working.

Starting in 1927 with a flock of White Leghorns which were quite susceptible to this disease (10 per cent survived the fowl typhoid disease induced by 12 million organisms of *S. gallinarum*), Lambert (1931 to 1936) was able to select progressively more resistant chicks. In four generations of progeny, resistance of each generation increased until an asymptotic value was reached at 80 to 90 per cent survival to this dose and for the large numbers of chicks tested at each generation. In this study three different resistant strains of chickens were established, each differing somewhat in their resistances. At the same time the work also showed that birds of one of our domestic breeds were almost uniformly susceptible in each generation's yearly tests when exposed to the 12-million organism dose. These strains of fowl have been carried on by J. Stadler and myself. During the period from 1937 to 1960, the genetic resistances established in these strains have been maintained without further contact with the disease. The course of the changes with generations of selection for resistance is shown in FIGURE 1 along with similar changes observed in another spe-

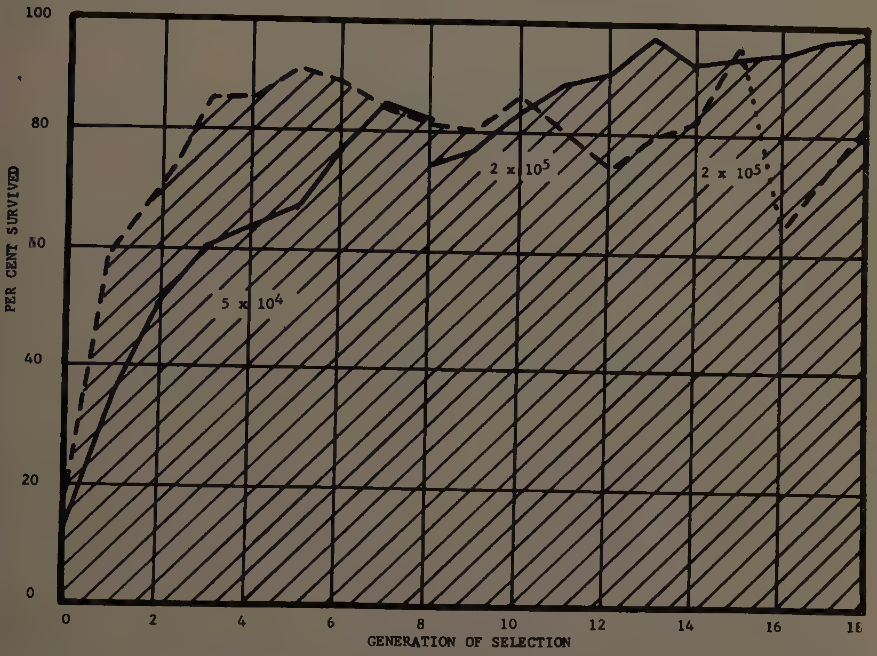

FIGURE 1. Progressive advance in resistance of the domestic fowl to fowl typhoid, *S gallinarum*, dose 12 million organisms, with selection of resistant birds as the parents for the following generations. Data from a similar study on mice and mouse typhoid *S. typhimurium*, dosages 50,000 and 200,000, are included for comparative purposes. Dashed-dashed and dot-dot lines are for fowl changes in resistance with selection. Solid line for mouse typhoid selections for resistance.

cies, mice, which were selected for resistance to another disease, mouse typhoid, where the initiating agent is *S. typhimurium*.

The resistance changes established through the selections within the different strains have been maintained to date. They may be considered as essentially permanent attributes of the strains. Three strains of resistant chicks were established. Crosses between them were made in all possible ways. The survival values within the groups were comparable among the crosses and also among their controls (TABLE 1).

These results showed that crosses among the resistant strains differed little in resistance. They had slightly more resistance than their parent inbred strains. There were no obvious maternal effects on resistance.

Table 1

Resistance of Chicks of Three Genetically Selected Lines to 12 × 10^6 *S. gallinarum* Inoculated at Seven Days of Age, Crosses Between Lines and Within the Selected Lines

Crossbred birds			Inbred strains		
Strain matings	No. of chicks	Per cent survived	Strain	No. of chicks	Per cent survived
A × B	66	97	A	66	96
A × C	58	88	A	92	95
B × A	18	100	B	97	96
B × C	58	91	B	134	96
C × A	37	84	C	183	76
C × B	64	84	C	160	73
Total and average	301	91		731	88

Inheritance of Resistance

Hybrid progeny between the resistant strains and the susceptible strains were obtained and tested by Lambert. The tests for resistance were by intraperitoneal injection of 12 million organisms of *S. gallinarum* at 7 days of age. The resistance of the different crosses is seen in TABLE 2.

TABLE 2 shows that resistance was reduced by crossing resistant birds to susceptible birds. No differences existed for the reciprocal matings, showing that maternal effects on resistance were absent for this disease. The crosses indicated the greater dominance of susceptibility in the phenotypes. The effects of the genotypic selection were carried through to the F_1 generation. The backcross chicks still further indicated the segregation of the disease resistant genes (TABLE 3).

The results confirm the segregation of the genes for resistance. Crosses of the hybrids to susceptibles lowered resistance. Crosses to resistance raised resistance of the resulting chick. The backcrosses were intermediate in their resistance between the susceptible strain and the F_1 in the first case and between the F_1 and resistant strain in the second case, as anticipated if the genes were segregating in the manner expected if resistance and susceptibility under the same environmental conditions were dependent on particular gene pairs. The number of the gene pairs was apparently large, and any average dominance there was contributed toward making the heterozygous chicks more nearly like the susceptible parent.

Genetics of Host Resistance and Susceptibility for Mouse Typhoid S. typhimurium in the Mouse

Confirmatory data for the inheritance of different levels of resistance to the bacterial disease mouse typhoid *S. typhimurium* has been obtained for the mouse (Gowen, 1960). Ten different strains have been established. Tests of these strains from 1944 to 1956 covering about 21,000 mice have shown the strains to vary in resistance from 84 per cent to nearly complete susceptibility, 0.2 per cent resistant. The resistance of the strains has been relatively constant for each year over the 11-year period despite the yearly and seasonal variations. The S strain has had 84 per cent survival, RI 68, K 51, Z 34, C 30, E 24, N 17, L 10, Q 1.7, and Ba 0.2 per cent. The disease resistance exhibited was dependent on the strain's innate natural resistance since each has had no known previous contact with the disease for at least 15 generations prior to the actual tests. Further tests for the absence of mouse typhoid contacts have always resulted in lack of *S. typhimurium* in cultures of liver, kidney, and spleen and of agglutinins in the blood. The pathogen *S. typhimurium* 11C (a line that when contrasted with other isolations of this species is in the high-virulent class) has been used throughout the tests.

TABLE 2

RESISTANCE OF HYBRID CHICKS FROM RECIPROCAL CROSSES OF STRAINS SELECTED FOR RESISTANCE MATED TO THOSE SELECTED BECAUSE OF THEIR SUSCEPTIBILITY

Parents			Progeny tested	Per cent survived
Male		Female		
Susceptible	×	Resistant	89	32
Resistant	×	Susceptible	160	31

TABLE 3

RESISTANCE OF RECIPROCAL BACKCROSSES OF RESISTANT AND SUSCEPTIBLE STRAINS

Parent types (male × female)	No. of chicks	Per cent survived
F_1 from susceptible male × resistant female		
F_1 × susceptible	84	5
F_1 × resistant	8	63
F_1 from resistant male × susceptible female		
F_1 × susceptible	160	22
F_1 × resistant	177	64

The host strains showed other characteristic differences in the manner in which they reacted with the disease. The S mice, although the most resistant, had characteristic differences in the sexes: only 74 per cent of the males as contrasted to 95 per cent of the females survived the tests. The difference was due to the males' much higher frequency of death 1 to 4 days following infection. The frequency curves of deaths showed pronounced and characteristic differences in their forms when compared visually or when contrasted by their means, modes, standard deviations, skewness, kurtosis, and curve types. The observed stratifications of the host resistances furnished evidence for the multigenic nature of disease resistance. Equally numerous physiological reactions to the disease would be expected in the hosts. The character basis for the disease as influenced by the genes would not be expected to be the same in the different strains. As will be shown later this was found to be true.

As with crosses between susceptible and resistant strains of chickens, the transmission of resistance to *S. typhimurium* in mice behaved as expected on genetic grounds. Convincing evidence for the genetic dependence of infectious disease resistance and susceptibility came from the repetition of the test results for the different strains generation after generation and year after year. The crosses between the different strains gave further support to these views. The results for these F_1 crosses are shown in FIGURE 2.

FIGURE 2 is based on a combination of data taken on 3 inbred strains resistant to mouse typhoid, 2 strains that are highly susceptible, and the possible hybrids and backcrosses between them. The initial contact with the disease organism *S. typhimurium* 11C (200,000 organisms) was made at 60 days of age.

Crosses within the inbred strains selected for resistance showed essentially the same high resistance to the disease that experience has led us to expect from the within-family tests. There was no noticeable increase in resistance due to any heterosis that might result from these crosses. Similarly crosses within the susceptible inbred strains gave the highly susceptible progeny that were observed in the within-family data normally collected.

The F_1 hybrids between the resistant lines (FIGURE 2, column 3) were somewhat less resistant than the progeny resulting from matings within the lines. The difference was not large, 8 per cent. The two succeeding crosses were made reciprocally. They were between highly resistant and highly susceptible strains of mice. There was no evidence favoring any influence of the mothers on the resistance of their offspring. In consequence the data were combined and are shown in column 4 of FIGURE 2. The results favored the genetic control of the disease severity rather than passage of any humoral bodies from the mothers to their offspring. In fact, the lack of any maternal effects also eliminated many possible environmental causes of resistance that have been hypothesized in the past as influencing disease resistance. Genetic factors alone emerged as the significant elements. The data also showed that the mice that had both parents from the resistant lines were no better in resistance than those that were highly heterozygous for resistance through having one parent resistant and the other highly susceptible. Dominance of resistance was indicated.

The crosses between the susceptible strains were interesting indeed. These strains, as shown by the second column of FIGURE 2, were highly susceptible to the disease yet the progeny derived from crossing these lines (column 5) were noticeably more resistant. On a genetic basis these results indicated that the susceptible lines did not carry the same genes for susceptibility, thus allowing the genes for medium resistance reciprocally to cover up some of the susceptibility genes so that the crosses displayed more resistance than might be expected at first thought. The fact that crosses between strains, continuously highly susceptible throughout a long period, resulted in progeny of more favorable resistance also gave evidence for the multiple-factor nature of resistance to this disease.

Backcross data gathered from these strains by Hetzer (1937) while in this laboratory further supported these views. The backcrosses were made to the susceptible strains and were made reciprocally. The results are incorporated in column 6, FIGURE 2. Reference to the resistance of the F_1 mice as shown in column 4 (resistant × susceptible) shows that they were highly resistant. Column 2 shows that the mice of inbred susceptible strain were highly susceptible. The backcrossed progeny were all intermediate in resistance as expected for the segregation of genes for resistance and susceptibility of the F_1 individuals.

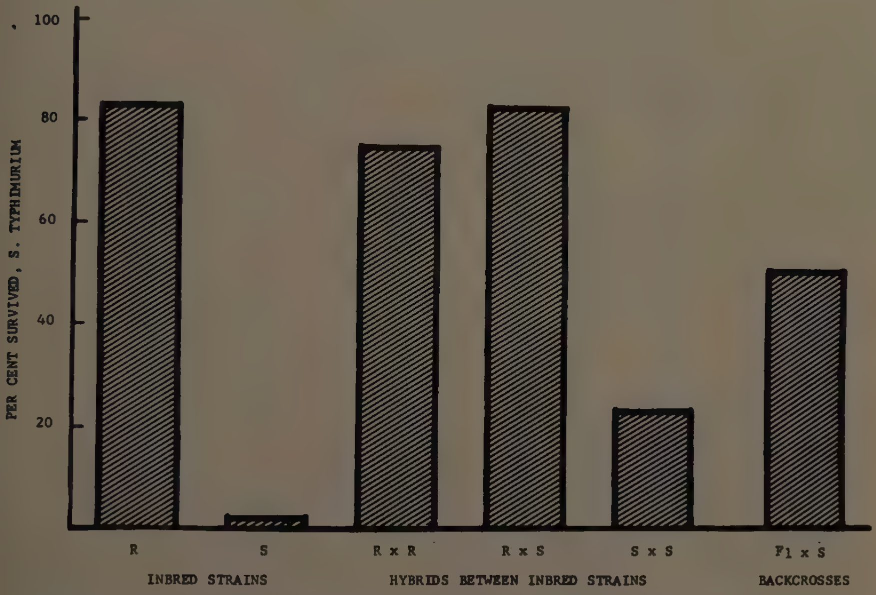

FIGURE 2. Survival of parental, F_1, and backcrossed strains of mice susceptible and resistant to mouse typhoid *S. typhimurium* 11C, 200,000 organisms.

These data on the inheritance of resistance and susceptibility to *S. typhimurium* in the mouse were comparable throughout with those for the domestic fowls and the disease, fowl typhoid, *S. gallinarum*. Tests on the inbred parents showed several genetical grades of resistance to these diseases that were established in the respective inbred strains. Hybrids between the strains and backcrosses of the F_1 hybrid animals to those of the susceptible strains supported the conclusion that, for each species, resistance to these diseases depended on genetic factors transmitted in the long-established manner. The effects of these genes were specific, pointing to their acting on different physiological processes that formed the integrated complex that made for resistance or susceptibility of the particular animals as well as host-specific similarities and differences that connected and separated species. As will be seen later, the modes of action of the host inheritance complexes were capable of expressing widely different phenotypes when interacting with diverse genetically controlled constitutions observed in genetically separated different infecting bacterial lines.

Multiple-factor Nature of Host Disease Resistance to Different Diseases

The multiple-factor nature of host resistance to a single disease was supported from other evidence: (1) identical twins in human diseases; (2) the consistency of the disease reactions of successive mice within inbred strains to mimic in a real sense the identical genotypes of identical twins; and (3) fulfilling the genetic expectations for disease susceptibility or resistance by mice from identical litters but of two different genotypes as derived from double matings in which female mice susceptible to the disease were mated individually in the same estrus period to two males: one of their own susceptible strain and the other of a resistant strain (Gowen and Schott, 1933*b*). Genetic information on the inheritance differences, such as coat color, was utilized to separate the two genotypically different types of progeny within the litter of the single mothers. Disease tests showed that the progeny types behaved according to their genetic patterns but not as expected for the identical environments in which they developed.

The same principles carried over to diseases having different etiological agents. Results of all studies comparing the disease resistance of individual genetic strains to several diseases indicated that there was no single factor or over-all constitution that was effective in resisting diseases having different initiating pathogens (Gowen, 1948).

In much of the earlier medical thinking, resistance to one disease was held to be indicative of resistance to another disease having different etiological origin. A person was held to have a good constitution who seemed resistant to many diseases as differentiated from a person who passed through attacks of a variety of diseases. Basically this concept would indicate that the inheritance was acting on an over-all resistance capable of affecting many diseases. Genetically, resistance to all or most diseases would be unlikely. This concept was tested in our laboratory a number of years ago by treating the same strains of inbred mice, S, WF, and L, with three different disease-causing agents: (1) *S. typhimurium*; (2) pseudorabies virus; and (3) the antigenic poison ricin (Gowen and Schott, 1933*c*). The strains of mice treated with typhimurium were in one case highly resistant S, and in the other cases WF and L were highly susceptible.

On the basis of a general over-all constitution it would be expected that the S mice resistant to the typhoid organism would carry over this resistance to the pseudorabies disease. This was found to be untrue. The S mice were most susceptible to the pseudorabies disease, whereas the WF and L mice were quite resistant. Similarly, the silver mice L were found quite resistant, the S mice medium resistant, and the WF mice were highly susceptible to ricin, a chemical agent of disease. The three mouse strains and three different diseases showed all possible combinations of resistance and susceptibility. The inheritances required for the resistance or susceptibility to these three diseases were evidently independent of one another. Similar information has been collected on other diseases (Webster, 1933; Schütze *et al.*, 1936) such as Pasteurella, louping ill, and St. Louis encephalitis; or again, *S. typhimurium*, Pasteurella, and Klebsiella pneumoniae; or *S. typhimurium*, *S. enteritidis*, louping ill, Pasteurella, and pneumococcus. The results of these tests agreed with the earlier work in showing that a resistant constitution for one disease is likely to be indicative of resistance to another disease only if the two diseases are fairly closely related in a taxonomic sense. When the diseases are of different types, resistance to one tends to be independent of resistance to another. The genetics of resistance conforms with that for the inheritance of other genetic characteristics that are dependent upon many genes in that some genes are independent, some linked, and some develop interrelated physiological effects. These results indicate that constitutional differences found in human or animal populations are made up of many reactions that are ultimately separable and therefore specific in action and in their inheritance. Breeding experiments confirm these expectations since it has been possible to form in mice four strains that illustrate all possible combinations of resistance and susceptibliity to the diseases caused by *S. enteritidis* and the virus of St. Louis encephalitis (Webster, 1937).

Phenotypic Basis for Disease Resistance

The stratifications of resistance observed for the different strains may be interpreted as due to a number of genes affecting different morphological, physicochemical, and immunological processes within the different hosts in relation to their response to the genotypes of the bacterial pathogens (Gowen, 1952). The inheritance differences suggest that resistance is probably due to several different characteristics and that no one attribute is all-important. A study of our strains suggests some factors that may be important as well as others that may have little relation to the natural resistance to the disease.

Since the strains before contact with the disease showed no detectable agglutinins or precipitins, it was clear that the natural resistance of these animals did not depend upon the presence of humoral bodies of the Ehrlich type. The absence of these products indicated that they were not formed in these mice before direct contact with the bacteria. Titrations of the bloods of mice infected with *S. typhimurium* showed that detectable agglutinins did not appear in the blood until after the disease has passed its peak for any given strain (Weir, 1942), again indicating that they had little part in natural resistance.

Body weight prior to the attack also seemed to have little influence on the disease outcome. Spleen weight increased remarkably during the early course of the disease, but strains differed in the degree of enlargement of this organ when the spleens were weighed on successive days during the disease course. Quantitative changes in size were not correlated appreciably with the differences in the strain's resistance (Gowen, 1954).

Strain characteristics extended to notable differences in the size and appearance of the internal organs. Differences in heart, kidney, liver, spleen, and testis weights were studied for their relation to the disease condition (Grahn, 1952). It was found that the genotypes of the respective strains accounted for about 40 per cent of the total variation in the weights of these organs. The kidneys were found to have approximately another 40 per cent of the weight differences accounted for by the genetic differences in the sexes. The livers, spleens, and hearts were essentially unaffected by the sex differences. When correlated with disease resistance, it was found that larger sizes of hearts, kidneys, and livers were related to the abilities of these strains to survive the disease attack. The masses of the hearts and livers were more indicative of resistance than the relative sizes of these organs. For the kidneys both absolute and relative weights were favorable to the animal's resistance. The spleen weight/body weight ratios had a high negative correlation with resistance of five strains, but this relation was shown to be specific since the most resistant S mice did not follow the trend.

Both resistant and susceptible mice lost weight during the four days following infection. Normal body weight for age was regained by the resistant mice, generally by the seventh day. These weight changes had an important bearing on the strain's natural resistance, but they may be considered as a result rather than a cause of the resistance. Circulating blood cells showed real differences for the different strains. The blood volumes of the mice varied over a rather wide range. Plasma volumes also showed a tendency to rise in certain strains, the rise being accompanied by a fall in the albumen and serum proteins. The serum proteins had unique characteristics. Of the strains analyzed only the E strain was observed to have a particular beta-1 globulin. The concentration of this protein was sufficient to account for the increased serum protein value found in this strain. During the course of the mouse typhoid disease this beta-1 globulin increased in value. Inheritance of this protein was studied in crosses of mice infected with the typhoid disease. The beta-1 peak was present in all sera, indicating dominance of the gene or genes responsible for this globulin. Similarly a new protein of a different type, alpha-1 globulin, was observed only in the sera of the S mice. Differences in the gamma-globulin content of the sera were likewise found to be strain-dependent (Thompson, 1952; Thompson *et al.*, 1954; Bowen *et al.*, 1957).

Cellular Factors for Mouse Typhoid Resistance

Studies on the blood cells of the different strains showed that the average leukocyte number increased as the resistance of the strains to mouse typhoid increased.

The two most resistant strains, the S and the RI, had the largest numbers of leukocytes. In one set of data the numbers were greater (Gowen and Calhoun, 1943) in the RI strain than in the more resistant S. In the other set of observations (Thompson, 1952) the S showed somewhat higher numbers (Gowen, 1954). The two sets of observations differed, the latter set showing fewer numbers of leukocytes per cubic millimeter of blood than the earlier material. However, they were essentially parallel as far as relative effects were concerned. The two strains that were intermediate in their resistance to *S. typhimurium* showed intermediate numbers of leukocytes. The most susceptible strains showed the smallest numbers. The strain differences in these cells were correlated with changes accompanying the course of the disease. The bloods of all strains showed a reduction in leukocyte number as the disease became more acute, the greatest change coming on the second day. Strains with the most numerous leukocytes retained their initial advantages throughout the course of the disease. Recovery in leukocyte numbers accompanied progress toward recovery from the disease and was time-related in that the most resistant strains recovered first.

Other phagocytic cells, such as the macrophages of the liver, displayed differences in their reactions to these bacteria (Oakberg, 1946). In resistant strains bacteria were seldom found within the macrophages. In fact, to obtain evidence for their phagocytosis it was necessary to inoculate the resistant animals with 100 times the dose that was given the more susceptible strains. Under these conditions a few bacteria were observed in the macrophages, but these bacteria did not stain well and their cell walls were rough in appearance. The bacteria gave all evidence of degenerating. The bacteria in the macrophages of the susceptible strains, however, were smooth in outline, well stained, and gave the appearance of reproducing normally. It would appear as though the susceptible mice were unable to destroy the bacteria when ingested into their macrophages.

Liver cells of the resistant as contrasted with those of the susceptible strain showed marked differences in their reactions to the disease. The susceptible mice died without pronounced clinical damage to this organ. Histological examination showed that the liver was depleted of glycogen and that the fat was not of normal chemical structure as indicated by staining with Sudan III. The resistant mice, on the other hand, showed large lesions of the liver, but these lesions were walled off from the rest of the normal tissue, which performed its customary physiological functions of storing glycogen and making normal fat.

The cellular nature of natural resistance was further brought out by our studies on the effect of X-ray irradiation on these different strains (Stadler and Gowen, 1957*a*, *b*, and *c*). By irradiating any particular regions of the body, the physiological functioning of the cells and organs in these regions was directly affected without interference with that of other regions. In an experiment on nearly 7000 mice of 5 different strains Stadler exposed the head third, middle third, rear third, head-mid, head-rear, mid-rear, and whole body to different dosages of X-ray irradiation. The data showed that instead of the body acting only by specific organ parts it also had a body-as-a-whole function. The mice were irradiated and allowed 15 days to express the radiation effects, the dosages being 320, 480, and 640 roentgens. The survivors were then exposed to *S. typhimurium* 11C to evaluate their natural resistance. Resistance was decreased when the mice were exposed to higher radiation doses. Loss of resistance was correlated with the natural resistance of the strain. Least-square analyses for the changes in resistance showed that irradiation to the mid-region, followed by that to the head, was most effective in reducing resistance although these effects were not large. Quantitatively the greatest factor for recovery was the presence of any unirradiated cells within the body, wherever their location might be. These results suggested that cells, even though they were extensively differentiated, retained many of their attributes that were not particularly related to this differentiation but that were capable of being activated on need.

Experimental Analyses of Genetic Determinants in Pathogen-initiated Infectious Disease

Up to this point attention has been focused on the effects of inheritance on the host in relation to disease conditions. Bacteria also have their own genotypes that may be separated into pure breeding lines within any bacterial species. Bacteria are, moreover, subject to mutations, which with their high numbers of separate organisms make appreciable effects on the disease patterns. Illustrations of colony differences and other characteristics of these mutations have now become familiar to many workers in the field (Barber, 1907; Zelle, 1942; Gowen, 1945).

Brief as this evidence is, it shows that these bacteria and, by inference, other disease agents are just as (or more) diverse in their genotypes as the hosts on which they act. In some instances they may have more significance to the induced disease because they reproduce in large numbers and are capable of broad dissemination. The small rate at which mutations of existing genes occur and the rapidity with which individuals carrying such genes may replace those of the existing population may become of prime importance in altering the character of the disease produced. The pathogenic characteristics may be altered in the direction of either greater or lesser virulence. The effects of these changes on disease expression have been under study in this laboratory for some 30 years on diseases such as tobacco mosaic, corn wilt, typhoids of both mouse and fowl, and leukosis. These results show that mutation materially affects disease severity. The mutated forms by selection may be subdivided into true breeding types. The types of survival have close relations to the environmental requirements for survival.

Seventy metabolic mutants have been obtained: (1) spontaneously; (2) by ultraviolet treatments; and (3) by X-ray treatments. These mutants were isolated into pure lines that have been tested for the effects of the different types of mutation on the virulence of the parental line as contrasted with those of the mutants (Gowen *et al.*, 1953).

Different mutant bacterial lines showed significant differences in their virulence and in their immunizing capacities. These lines have greatly extended the known mutative changes in pathogenicity. The virulence of the mutant was related to the virulence of its parent type. A parent of high virulence tended to have mutants of lesser virulence; a parent that was avirulent tended to have mutants of greater virulence than the parent. The curvilinear correlation observed accounted for some 37 per cent of the observed variation in the active immunizing power of these mutants when the virulence was known. Parents having poor immunizing powers tended to have mutants that were slightly better immunizers. Similarly, parents having good immunizing powers tended to have mutants with somewhat less active immunizing capacity.

The virulence and immunizing capacity of the different nutrient-requiring mutants differed more than would be expected on random sampling. However, the variation within the metabolic mutants of like requirements was greater than that either among metabolites or within metabolites and lines. These differences were interpreted as indicating that the particular chemical changes observed in the mutants were not the primary cause of the variations observed in either virulence or immunizing capacity. The observed variation could be due to two factors: (1) mutations of other genes arose simultaneously with the particular metabolite mutant; or (2) the genes controlling a given metabolite requirement were found in several loci and these genes had multiple or different effects; the variation of any of these giving the metabolite requirement but with a virulence or immunity different from that of the other genes.

The mutant process that led to a line having a phenotypically different metabolic requirement from that of its parent (as studied herein) had no consistent influence on either virulence or active immunizing ability of the new bacterial line.

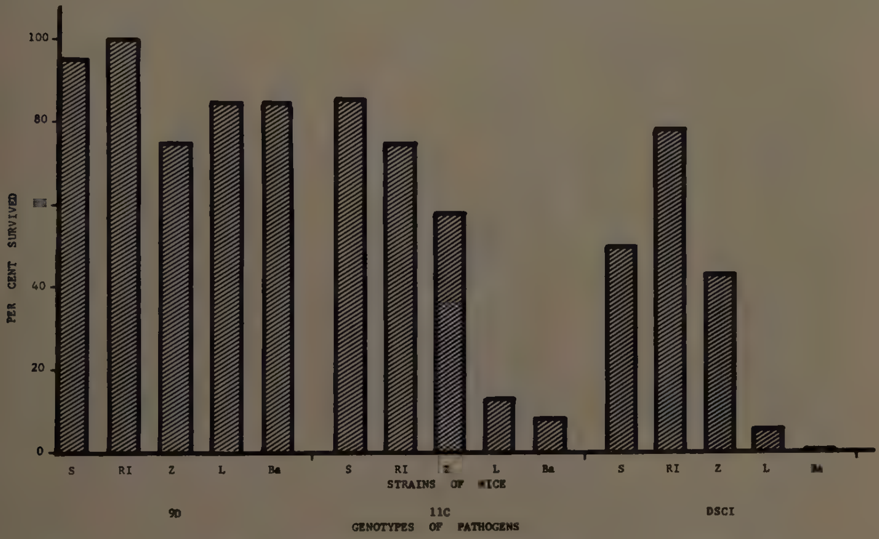

FIGURE 3. Comparison of lethality of mutants, 9D and DSC1 as derived from 11C, *S. typhimurium* for the host strains of mice S, RI, Z, L, and Ba.

Direct Virulence Mutations

In an earlier experiment in our laboratory, Weir (1942) extensively studied the effects of spontaneous mutation on the virulence of three particularly selected lines, line 9D being of low virulence, 11C having medium virulence, and our DSC1 having high virulence. Lines 9D and DSC1 had previously been established by Zelle (1942) from mutations he observed in a study of the selection effects of host resistances on the pathogens that might invade these hosts.

The averaged differences in lethality of the different mutants when introduced in the different host strains at the dose of 200,000 organisms is shown in FIGURE 3.

FIGURE 3 shows that the bacterial genotypes may mutate to different levels of lethality. Experience has shown that these levels generally may be maintained over many hundreds of cell divisions for each line. The phenotypes of the pathogens derived by mutation still affect the resistances of the host strains in an order similar to that observed for the parent bacterial line. Mutation may lead to pathogen lines that may be either phenotypically virulent or avirulent.

The characteristic lethal potentialities of the different mutant strains were retained when killed-bacterial cultures were the initiating disease agents. The comparative data were for pathogen lines 9D and DSC1 inoculated at dosages of 20 million killed organisms. Data on 11C were unavailable at these inoculation dosages, but at other dosages they showed that the host resistances were similarly influenced. Graphs for these data are found in FIGURE 4.

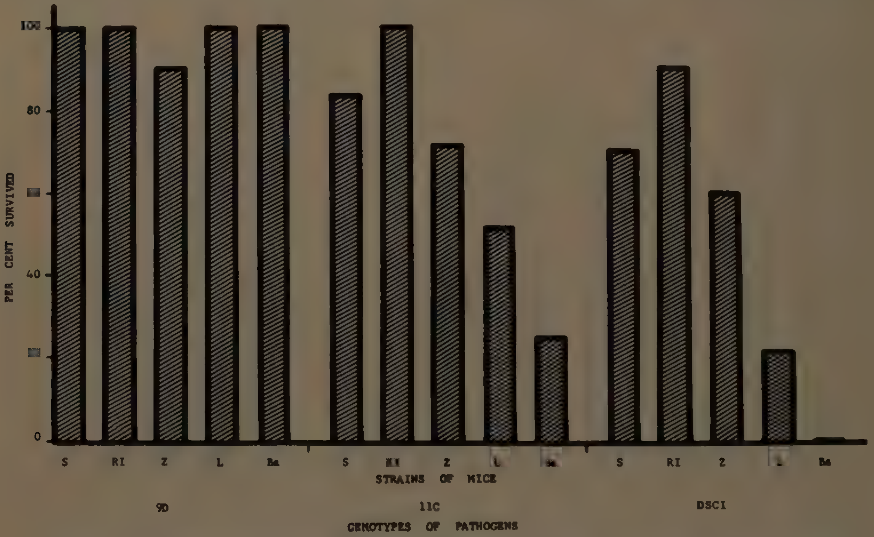

FIGURE 4. Resistance of mice of different strains to killed cultures of different lines of *S. typhimurium* derived by mutations from line 11C.

Clinically the killed cultures of *S. typhimurium* displayed disease syndromes in the different mouse strains similar to those of like live culture lines when inoculated at a dosage approximately 100 times less than the heat-killed cultures. The resulting lesions observed and the organs cultured, however, were sterile. Again the 9D line lacked pronounced virulence for any host strain. DSC1 killed cultures were virulent for the susceptible lines L and Ba, medium virulent for Z, and somewhat more virulent for the S and RI strains. The line 11C in killed culture remained more virulent than 9D and less virulent than DSC1 although the evidence for this fact must be derived from experiments with other inoculation dosages than those used in these experiments.

Natural Changes in Virulence of Pathogens

Similar virulence differences in strains of pathogens have been noted at various periods in man's history for diseases of bacterial and virus origins.

Like observations have been made for diseases of both domesticated animals and plants. They have occurred several times during our own laboratory work; some instances have been studied for their causative factors. In June of 1940 a highly virulent culture of *S. gallinarum*, which had been used extensively from November 1939, was transferred to new culture media and placed at near-freezing temperatures to reduce its rate of growth and new-media requirements. Five months later a descendent culture, having excellent growth, was removed from the cold, transferred to new culture media, and incubated at 40° C. Customary inoculation dosages were introduced into test chickens. The results showed that the culture had completely lost its virulence. Serological and cultural tests showed that the culture was *S. gallinarum*. The phenomenon was one that could be accounted for by a mutation of the original virulent to an avirulent type accompanied by a better growth potential under the culture conditions. More numerous progeny and better survival of the mutant had enabled it to replace the virulent form in the bacterial population, a view which our previous and also later experience has made likely.

The problem was to reverse the process and to obtain a virulent culture. When Stadler inoculated highly susceptible chicks with 2 billion organisms of this culture, no disease was observed. *Salmonella gallinarum* however was recovered from the cultures of the hearts, livers, kidneys, or spleens from 5 of these chicks. Two lines were soon lost. The other 3 lines were passed separately and blindly through 6 successive 10-week-old birds of the susceptible strain. The inoculating dose was 2 billion organisms, and the bacteria remained in the birds 1 week. At the seventh passage, virulence tests were made on 11 different 10-day-old chicks; line D7 killed 11 in less than 10 days; line C7 killed 6 out of 11 but took 21 days; line E7 killed 8 of 11 but also took 21 days. On extensive tests over a period of some years, line D has proved to be a highly virulent culture.

Planned experiments on larger numbers of chickens gave similar results. From the avirulent stock culture described above, 20 colony isolations were made. As this organism does not clump appreciably, each of these colony isolations probably represented the descendents of a single bacterium. Ten of these avirulent lines were exposed to the environment of our inbred, highly resistant chickens already described. These inbred lines are capable of surviving nearly 1000 times the number of bacteria that will cause death in most flocks. The other 10 lines of avirulent bacteria were grown in a strain of chickens marked by susceptibility to fowl typhoid. Two chicks were used at each passage for the resistant host line and 1 chick for the susceptible host line. The avirulent strains of *S. gallinarum* were thus exposed, on the one hand, to the intensely unfavorable environment of the resistant strain of host and, on the other hand, to the more favorable environment of the highly susceptible host (Gowen, 1951).

Each culture was passed successively through 16 different 10-day-old chicks. Despite the fact that twice as many chicks were available for recovering the organism at each resistance passage, 24 passages were lost in the transfers through the resistant host compared with 10 for the susceptible series. The avirulent line has great difficulty in establishing itself even to making a mild disease in the resistant host strain. This fact suggests that the resistant host would be a potent selecting force, tending to pick out the progeny of any variants characterized by increased virulence. This did not turn out to be the case.

Small tests were made throughout the passage experiments. When changes did occur they came suddenly during a single passage and resulted in a substantial gain in virulence, the total amount of change differing for different lines. The subsequent tests showed a retention of the new change in virulence. These results favored mutation and subsequent replacement of the avirulent type by the virulent mutant.

Tests of the surviving lines at the end of the 16th passage gave further support to this conclusion. Two of the lines, I and R, had not changed in virulence as the result of growing in their natural host for one-half year. One line was carried in the resistant host. The other line was passed through the susceptible host. If virulence is due to chance mutation, then essentially equal numbers of mutations would be expected in each group. Two lines of medium virulence were established from the resistant host against three lines for the susceptible host. Seven highly virulent lines came from resistant-host passage and six from susceptible-host passage.

Ten lines that passed through the resistant hosts showed virulence as follows: a dose of 100,000 organisms inoculated into 74 resistant chickens led to 20 per cent death; inoculated into 203 susceptible chickens led to 70 per cent death. With 100 millions of organisms as the dose, 70 resistant chickens had 22 per cent death; 124 susceptible chickens had 88 per cent death. For the lines derived by passage through the susceptible host, the 29 resistant chickens with 100,000 dosage had 7 per cent death, and the 186 susceptible chickens had 84 per cent death. For the 100-millions dosage, 54 resistant chickens had 22 per cent death, and 98 susceptibles had 86 per cent death. These data show that passage through either host is equally favorable to establishing virulence. The degree of increase in virulence may be judged by the fact that the original avirulent culture inoculated in a dose of 100 millions of organisms showed no death on the resistant host and only 34 per cent on the susceptible host.

Chicks of either strain are highly efficient selective agents favoring any variants toward virulence and encouraging them to multiply at the expense of the avirulent type. The population within the host becomes rapidly purified toward the virulent type. The genetic constitution of the domestic fowl, the natural host to this disease, is sufficient to create the necessary conditions for this selection process. The culture media, on the other hand, appears to favor those organisms whose genetic constitution is for a saprophytic type of growth.

Similar results were observed for *S. typhimurium* in mice in experiments conducted by Zelle (1942) in our laboratory. In essence, the different forms show changes in virulence to be analogous to mutations in higher forms. The rate of mutation for a given type is small. The changes are sporadic in their appearance and when they do occur, they are permanent and true-breeding. The variation can go in two directions: toward higher virulence or toward avirulence. The environment of the host or culture medium acts as a selective agent for the genetic type that fits the environment.

The data indicated that with environmental conditions favorable to select mutations toward either avirulence or virulence the established phenotypic changes observed in the pathogen were toward those adaptive to the environmental requirements. However, the organism itself may mutate either way. Phenotypical back mutations have been discovered and proven by us for 11C *S. typhimurium*. When sufficiently extreme, mutation may initiate disease having pandemic possibilities in spread and invasive power.

Synergetic Aspects of Mixtures of Pathogen Genotypes

Disease as it occurs in nature is the resultant of an intermixture of many divergent host and pathogen genotypes and their corresponding phenotypes in diverse and varying environments. This mixture of types may be simulated in experiments in which different pathogen genotypes form different proportions of the disease-initiating population. This question has been studied by both Zelle (1942) and Weir (1942) in our laboratory. The data of TABLE 4 give the lethalities of mixtures of 9D and DSC1 live cultures as contrasted with those for each genotype taken separately (Weir, 1942).

TABLE 4

LETHALITY OF TWO PURE LINES OF *S. TYPHIMURIUM* CONTRASTED WITH EQUAL MIXTURES OF THE TWO LINES

Live cultures

Strains	Genotype: 9D, 200,000		Genotype: DSC1, 200,000		Genotypes: 9D, 200,000 DSC1, 200,000	
	Ratio survived	Number tested	Ratio survived	Number tested	Ratio survived	Number tested
S	95	20	50	20	80	20
RI	100	20	79	19	90	20
Z	75	16	6	18	19	16
L	85	20	5	20	5	19
Ba	85	20	0	20	0	20

Heat-killed cultures

Strains	Genotype: 9D, 20,000,000		Genotype: DSC1, 20,000,000		Genotypes: 9D, 20,000,000 DSC1, 20,000,000	
	Ratio survived	Number tested	Ratio survived	Number tested	Ratio survived	Number tested
S	100	20	70	20	75	20
RI	100	20	90	20	92	26
Z	90	20	60	20	70	20
L	100	24	22	23	26	19
Ba	100	24	0	20	5	19

The data of TABLE 4 on the lethality of 200,000 introduced live organisms again brought out the relative avirulence of the 9D bacterial population as contrasted with the virulence of the DSC1 population. The mixture of the two genotypes at equal dosages was only about as lethal to the tested mice as the most virulent single culture DSC1 inoculated at the same dosage. Results for other population mixtures supported this view. When killed cultures containing 20 millions of bacteria were substituted for the live cultures, the observed lethality for the mixtures again were similar to those of the most virulent organisms and bore little relation to those of the avirulent component in the inoculum. The rapid eliminations of the avirulent type organisms would explain these results. Test of organism mixtures by Zelle (1942), where the virulent and avirulent organisms could be phenotypically identified, supported the elimination of any weakly virulent bacterial pathogens in mixtures with strongly virulent organism. These results favored the view that no synergetic supplementation of virulence beyond that of the most virulent organism takes place when organisms of the same species but of different genetic types were introduced into the same hosts.

Genetic Determinants in Immunity Acquired Through Vaccination

Immunity acquired actively plays an increasingly significant part in resistance to disease. The genetic influences are contributed by both the specific host and the specific pathogen. In the course of our experiments the significance of the host and pathogen genotypes and certain environmental factors have been measured for six of our inbred mouse strains and three of our *S. typhimurium* lines.

The host influence on immunity acquired through vaccination was measured for six inbred strains. The natural resistance of these strains has already been indicated earlier in this paper. The bacterial lines were all derived from the same source. The basic line is our virulent 11C. From this line, DSC1 and 9D were derived by mutation (Zelle, 1942). The line 9D initially showed 21 per cent fewer deaths than 11C under the same test conditions; DSC1 showed 16 per cent more deaths in the same experiment. Unfortunately DSC1 apparently back-mutated to lower virulence during the course of these studies. When used in the acquired immunity experiments, it was of somewhat less virulence than when originally isolated. The change appears due to a mutant replacing the parent bacterial line. However, in the mutant form the DSC1 still retained somewhat more virulence than 11C.

The genotypes of the host strains showed marked differences in response to vaccination when challenged by 50 millions of 11C organisms. The degree of immunity resulting from vaccination was dependent upon how these different genotypes interacted with the various factors involved. The factors chosen for study were host genotype, pathogen genotype, doses of vaccine, times vaccinated, and sex. The experimental design had 324 cells filled with a minimum of 15 mice each.

For this large challenge dose, fewer than 50 per cent of the mice were protected. Protection was greater for those mice receiving the vaccine prepared from the bacterial line having the most virulent genotype. Protection was also greater for mice receiving the largest amount of vaccine. The factorial nature of these experiments allowed unbiased estimates of the other variables. All of the major variables (host genotype, pathogen genotype, doses of vaccine, number of vaccinations, and sex) had highly significant effects on the vaccination resistance. From a genetic viewpoint 55 per cent of the total variation in vaccine-generated resistance was found to be attributable to the inheritance factors involved in the vaccination pattern.

Two of the mouse strains showed specificity in the manner in which they die when vaccinated and subsequently challenged. The S and RI males frequently died shortly after inoculation, apparently from rapid release of endotoxin. Deaths of the other mice followed the morbidity curve characteristic of that observed in the natural infection in which growth of *S. typhimurium* took place within the host and ultimately causes death.

Genetic Aspects of Disease

The genetic aspects of disease that have been considered in this paper may be summarized only by a consideration of the variables acting in multidimensional space. It has been shown that both pathogen and host species may be subdivided into relatively true breeding lines or strains, respectively, through genetic techniques. Environmental factors affect these reactions in nature. All three components are normally operating in the disease syndrome. However, in the laboratory it is possible to hold many of the environmental factors constant, allowing only the host influence and pathogen influence to vary in major dimensions. The third dimension may be used to measure the survival of the animals; the model is applicable to diseases of all types. Results in most cases are similar in pattern.

At one end of the model specific doses of the pathogen cause complete mortality to the hosts; in the middle the host genotypes with the same dose of highly virulent organisms have high, but not complete, mortality; at the end where highly resistant host genotypes are treated with virulent organisms, the disease may be recognized by morbidity but may cause no mortality. The highly virulent pathogens cause the greatest number of mortality cases in all classes of hosts. Those of lesser virulence cause varying degrees of mortality according to the genetic constitutions of the hosts into which they are introduced. The pathogens that are essentially avirulent cause little or no mortality in any hosts. The genotypes fit together in such a manner as to give all types of disease severity. This lock-to-key arrangement indicates that the factors necessary are specific in action.

In principle, the similar factors of inheritance determine the acquiring of immunity through a previous attack of the disease or through vaccination. Natural resistance to disease comes as a consequence of the factors of inheritance in the host and in the pathogen operating with equally numerous factors in the environment. One of these factors can claim no special advantages over the others. All elements have the potentialities of causing pronounced effects, the extent of which depends on the special situation. Understanding the detailed actions of these factors presents medicine with its greatest challenge as well as its greatest opportunity for progress.

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GENETIC FACTORS IN RESISTANCE TO MALARIA

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There is good evidence—some of which is reviewed in this monograph—that when a population of animals is repeatedly exposed to an infectious disease producing significant mortality, the progeny of surviving animals show through several generations increasing resistance to the disease. It would be generally agreed that this is a process of selection of genetically resistant animals. However, as a rule the genetic factors affecting resistance to disease are complex, depending upon many genes affecting antibody synthesis and other reactions. In the case of malaria in man, however, considerable genetic resistance is known to be conferred by two major genes, the sickle-cell gene and the glucose-6-phosphate dehydrogenase-deficiency gene; there is also strong circumstantial evidence that the same is true of other abnormal hemoglobin genes. The delicate interplay of these and other more complex genetic and environmental factors with acquired immunity to malaria will be discussed in this paper.

Natural History of Malaria in Holoendemic Areas

Most of this discussion will be concerned with malignant tertian malaria in areas where it is hyperendemic or holoendemic, that is, where *Plasmodium falciparum* is transmitted throughout the year by anopheline vectors. As shown in the accompanying map (FIGURE 1), this situation prevails over much of central Africa and southern Asia, including the East Indies. The intensity of transmission varies from area to area and from season to season. In some regions it has been shown that exposed individuals receive an average of one or more infected bites a night, but the transmission rate is much lower in certain areas at certain seasons.

The over-all pathological picture is the same in all these regions although the duration of each stage in the sequence varies,[1,2] being most compressed where transmission is intense. Newborn infants have low parasite rates and counts. A number of factors have been regarded as contributing to the apparent resistance of newborns to malaria, including: (1) transplacental acquisition of serological immunity from the mother; (2) the presence of a high proportion of fetal hemoglobin in circulating red cells; (3) low levels of *p*-aminobenzoic acid in milk diets; and (4) the fact that newborns are less exposed and less often bitten by mosquitoes than older children.[3]

In later infancy and up to two years of age, children show the most severe manifestations of malaria. If blood samples are taken from populations, the great majority of children in this age group shows parasites, and the parasite counts attain high levels (above 10,000 parasites per millimeter of blood). There is no doubt that malaria is responsible for considerable morbidity. Children often have fever and other signs and symptoms of the disease; nearly all have greatly enlarged spleens. During this period an appreciable proportion of children die—as many as one fifth of all children—but there is disagreement over the part played by malaria in this mortality. The problem is difficult to resolve because cases in which malaria can be shown unequivocally to be responsible for death (for example, cerebral malaria) are not often seen by physicians. There are, however, several ways in which malaria might be a critical contributory factor to deaths from other causes. Included among these is the leukopenia associated with chronic malarial parasitemia, which lowers resistance to pyogenic infections.[5] On the most conservative estimate,[6] malaria in holoendemic areas "causes a great deal of illness and mortality of some 10 per cent of infants." Infants also have high rates of gametocytes, from which the infection is transmitted to the vector.

Between one and five years of age the effects of acquired immunity become increasingly apparent. This is manifested by decreased morbidity in the presence of parasites, decreased parasite counts and, subsequently, decreased parasite rates (especially gametocyte rates), and decrease in the size of the spleen. As a rule, by the time the children are of school age they suffer little morbidity and have very low parasite counts. This is the state of hyperimmunity that persists throughout life when there is continued exposure to the parasite. The mortality from malaria in older children and adults in holoendemic areas is negligible. Africans show very high γ-globulin levels, a substantial part of the increase being due to malarial infection.[7]

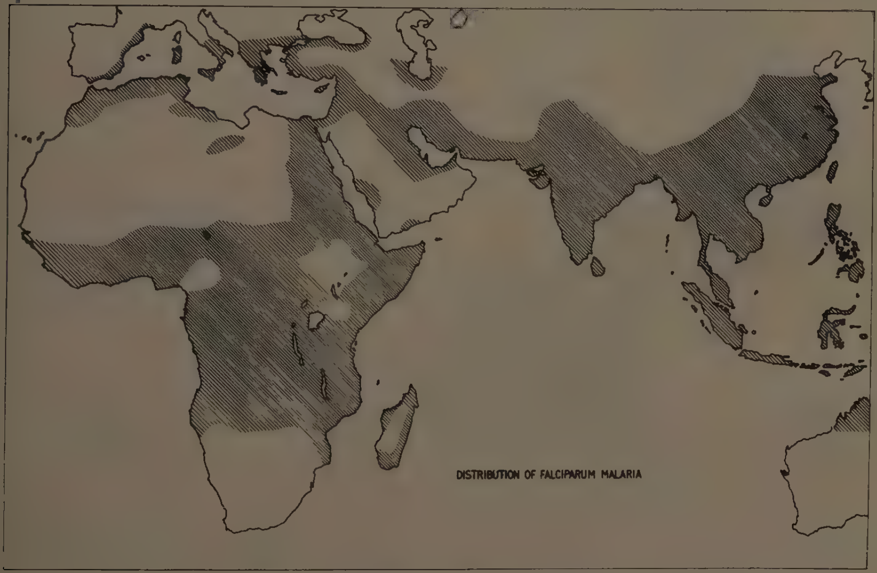
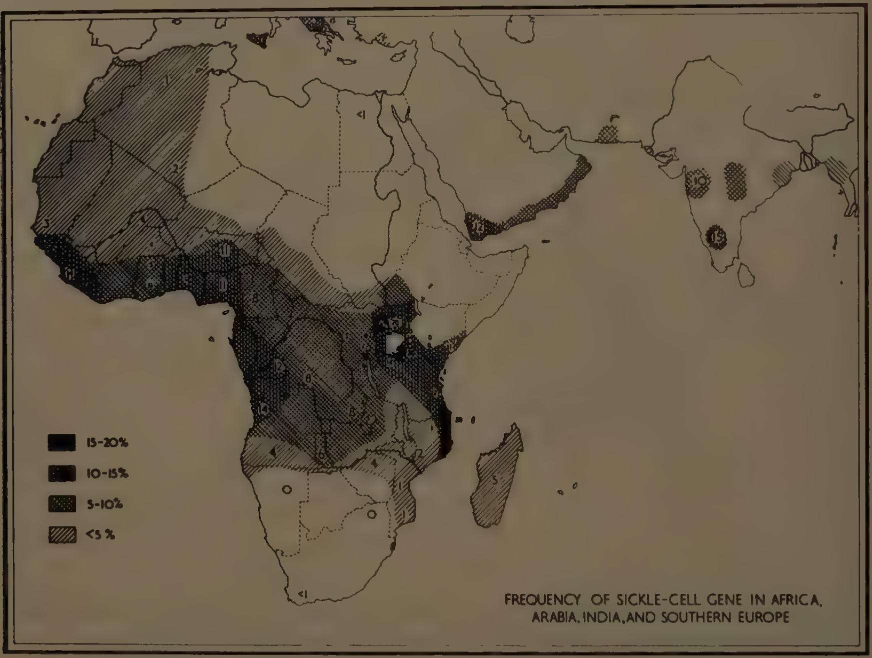


FIGURE 1. Distribution of *falciparum* malaria in the Old World (data of Boyd[1]).

The Sickle-cell Gene and Resistance to Malaria

The sickle-cell gene, which is common in some southern European and Asian and many African populations (FIGURE 2), is responsible for the production of an abnormal hemoglobin. Sickle-cell hemoglobin differs from normal only in the substitution of a valine for a glutamic acid residue in each half-molecule,[8] yet the properties of the two proteins are substantially different. When deoxygenated, sickle-cell hemoglobin molecules aggregate into rigid, rodlike masses that distort the red cell into the sickle shape. In sickle-cell homozygotes this happens at physiological partial pressures of oxygen, and so brings about anemia and vascular obstruction, whereas in trait carriers (heterozygotes) sickling takes place only at very low partial pressures of oxygen.[9]

Early work showing that sickle-cell trait carriers are resistant to *falciparum* malaria has been fully reviewed by the author.[3] The demonstration of resistance[10] was made in the field on groups of young children. The concept was challenged by workers who, failing to understand the factors mentioned in the previous section, attempted to refute it on the basis of observations on schoolchildren and adults. Later investigators turned their attention again to young children and were able to confirm the original findings in detail. The following conclusions in the review[3] were justified by 1957:


15-20%
10-15%
5-10%
<5%
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FREQUENCY OF SICKLE-CELL GENE IN AFRICA, ARABIA, INDIA, AND SOUTHERN EUROPE

FIGURE 2. Frequency of the sickle-cell gene in aboriginal populations.

(1) In children under one year of age who are highly susceptible to *P. falciparum*, as high a proportion of sickle-cell trait carriers show parasites as subjects without the sickle-cell trait. It was inferred that in sickle-cell trait carriers the infection continues for the normal length of time—which may be important in building up immunity—but that the presence of sickle-cell hemoglobin limits the multiplication of trophozoites so that the parasite counts remain low. Since the mortality from *falciparum* malaria is closely related to the height of the parasite count in peripheral blood[4] (FIGURE 3), it was inferred

that the sickle-cell trait carriers would have a greater chance of surviving through the particularly dangerous stage of early exposure to malaria than would children without the trait.

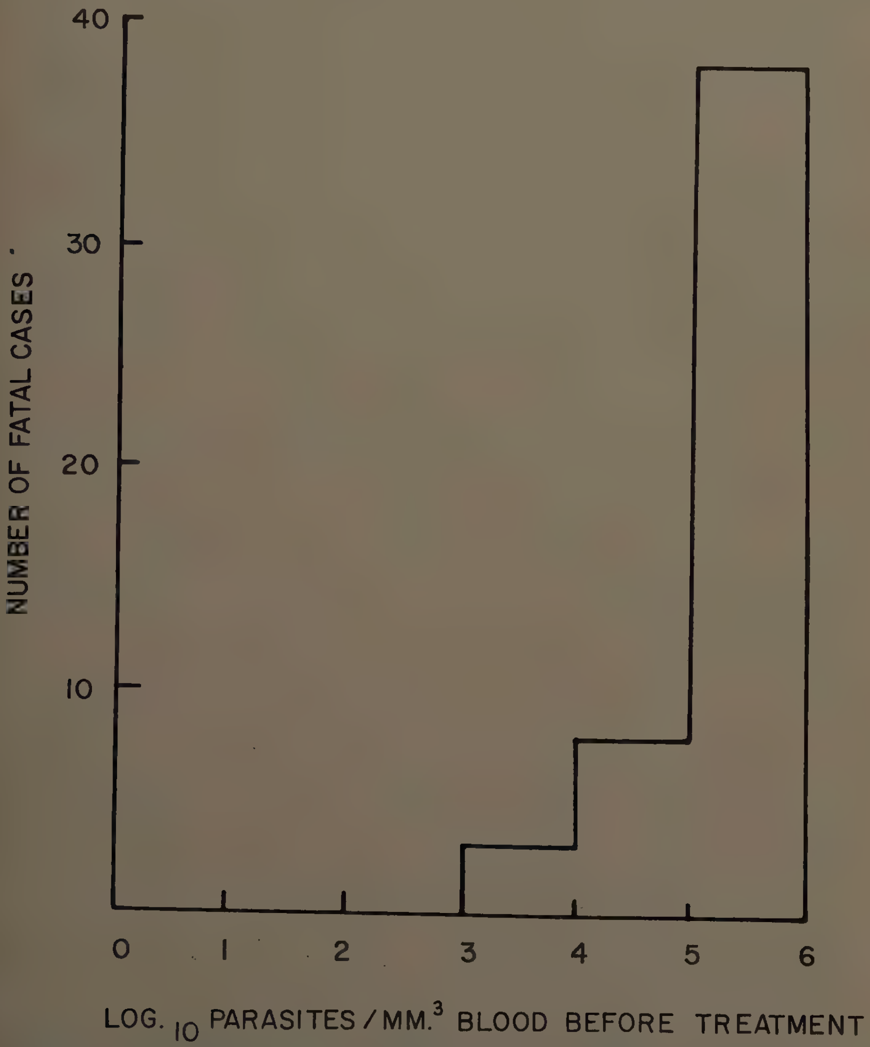

Figure 3. Number of fatal cases of *falciparum* malaria in relation to the height of the parasite count in peripheral blood before treatment (data of Field[4]).

(2) While immunity to malaria is building up in the one- to four-year-olds, the effect of the sickle-cell trait is even more noticeable. The proportion of subjects showing parasites, as well as the parasite counts, is lower in sickle-cell trait carriers (figure 4). It seems reasonable to conclude that *falciparum* infections are of shorter duration (they are also associated with lower parasite counts) in trait carriers than in those subjects without the trait. This obser-

vation and the significantly lower incidence of cerebral malaria found in sickle-cell trait carriers[11] again suggest that mortality from malaria is likely to be higher in subjects without the trait than in trait carriers.

(3) In subjects who are highly immune, the effect of the sickle-cell trait on parasite rates and counts is negligible; the effects are overshadowed by those of acquired immunity. These conclusions have been fully borne out by further work. Vandepitte and Delaisse[12] found that in 496 Congolese children under the age of 10 years suffering from malaria: (1) the proportion of sickle-cell trait carriers was significantly lower than in the general population; (2) high parasite counts occurred predominantly in nonsickling children; and (3) the mortality from malaria was greater in normal than in sickle-cell trait children. Similar observations have been recorded from elsewhere in the Congo,[13] Uganda,[14] and Nigeria.[15]

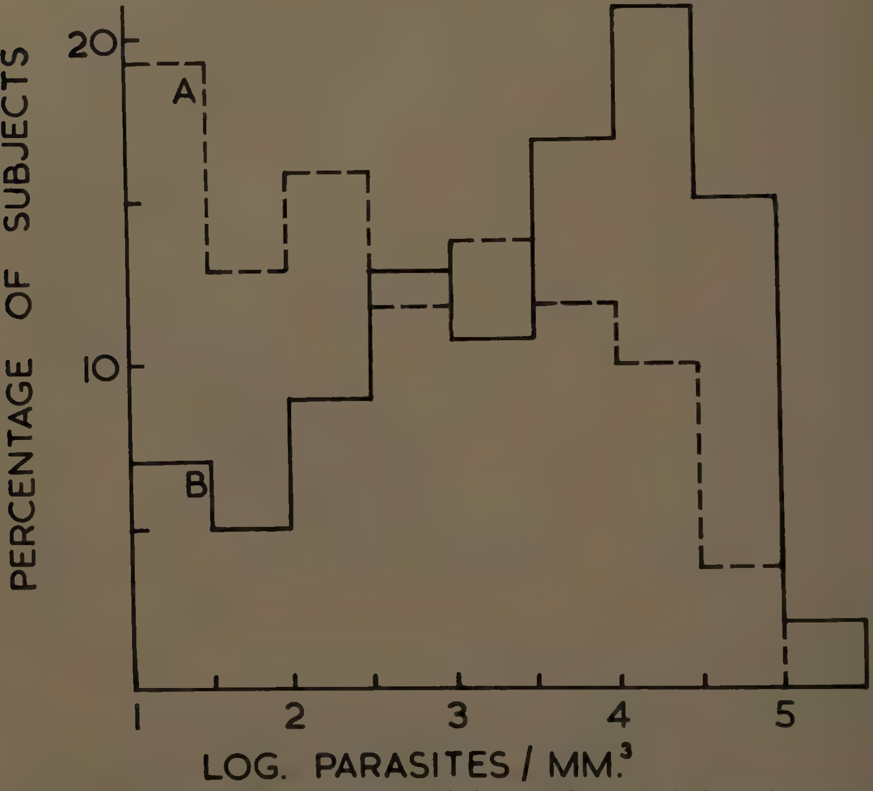

FIGURE 4. *Plasmodium falciparum* density in infected children with the sickle-cell trait (A) and without the trait (B). Combined data of Allison[10] and of Raper[11] for Kampala, Uganda.

The suggestion[3,12] that the amount of sickle-cell hemoglobin might impose on the intracorpuscular parasites a certain maximal level beyond which they could not multiply is borne out by observation (TABLE 1). The maximum level is lowest when all the hemoglobin is of the S type (in sickle-cell homozygotes), intermediate when only some of the hemoglobin is of the S type (heterozygotes), and highest in the absence of hemoglobin S (normal subjects).

Possible Mechanisms of Resistance of Sickle-cell Heterozygotes to Malaria

Although there is now general agreement that the sickle-cell trait confers considerable protection against malaria, the mechanism by which it does so is still undetermined. A number of possibilities have been considered. Mackey and Vivarelli[16] suggested that the presence of malaria parasites in cells of heterozygotes might cause them to assume the sickle shape and then to be removed from the circulation by phagocytosis. Miller *et al.*[17] have pointed out that parasitized cells tend to adhere to the walls of blood vessels; they suggest that parasitized red cells adhering to the walls of capillaries and veins might become sickled and so interfere with the multiplication of the parasite. It is, in fact, possible that malaria parasites, having a high rate of oxygen uptake, might induce sickling of cells even though the oxygen tensions in mixed venous blood are not low enough to induce sickling of the cells of heterozygotes.[9]

TABLE 1

MAXIMUM *P. FALCIPARUM* PARASITE COUNTS PER MILLILITER OF BLOOD IN AFRICAN CHILDREN UNDER TEN YEARS OF AGE

Locality	Hemoglobin type			Authors
	S (+F)	A + S	A	
Luluaborg, Republic of the Congo	11,500	250,000	1,050,000	Vandepitte and Delaisse[12]
Kampala, Uganda	10,000	160,000	800,000	Raper[14]

Allison suggested that *P. falciparum* might be unable to break down and utilize sickle-cell hemoglobin as effectively as normal adult hemoglobin. *Plasmodia* metabolize hemoglobin very efficiently in the intact red cell.[18,19] The first stage of the process appears most critical, and denatured hemoglobin is more readily utilized than the native protein.[20] Recent electron micrographs[20a] show that *Plasmodia* take up large quantities of hemoglobin by pinocytosis. Hemoglobin in an intracellular environment is likely to be rapidly deoxygenated; this is especially true of sickle-cell hemoglobin that appears to have—contrary to a previous report—a lower affinity for oxygen than normal adult hemoglobin.[20b] Furthermore, if the cytoplasm of malaria parasites resembles that of phagocytic cells,[20c] the intracellular hemoglobin will be exposed to acidic conditions, under which denatured sickle-cell hemoglobin is much less soluble than normal adult hemoglobin.[20d] However, there is no doubt that *P. falciparum* can grow, produce pigment, and divide in the cells of sickle-cell hemozygotes, in which 80 to 100 per cent of pigment is of the S type. Raper[14] was unable to find any difference in the rates of parasite growth in the cells containing A + S hemoglobin and A hemoglobin alone when cultivated for brief periods *in vitro* up to a single schizogony cycle. These results make it unlikely that there is an absolute inability to metabolize sickle-cell hemoglobin, but even a relative disadvantage might be decisive during some critical stage such as schizogony.

The reason for the sickle-cell heterozygote's resistance to malaria is therefore uncertain but the indirect evidence suggesting that thalassemia and other abnormal hemoglobin traits also protect against malaria implies that explanations not taking into account the mechanical effect of sickling still deserve consideration.

Malaria and the Glucose-6-phosphate Dehydrogenase-deficiency Trait

It has been established[21] that about 11 per cent of United States Negro males have an intrinsic red blood cell abnormality. A striking feature of the abnormality is the low activity of the enzyme glucose-6-phosphate dehydrogenase, which is responsible for the first and rate-controlling step in the hexose monophosphate shunt metabolic pathway. The primary genetically controlled abnormality does not appear to be a defective synthesis of the enzyme itself but the absence of a stromal factor that activates the enzyme.[22] The defect is inherited as a sex-linked character with full expression in hemizygous males and homozygous females and partial expression in heterozygous females. Persons with the defect are liable to develop severe hemolysis: (1) on exposure to therapeutic doses of the antimalarial 8-aminoquinolines (such as primaquine and pamaquine) and certain other drugs; (2) on ingestion of broad beans (*Vicia faba*); and (3) when they have certain virus diseases.[23]

Motulsky[24] and Allison[25] have pointed out that the distribution of the glucose-6-phosphate dehydrogenase-deficiency trait corresponds with that of malignant tertian malaria. (FIGURE 5). The local distribution also favors this view: in east Africa and Sardinia some areas are (or were until recently) very malarial; others are free from the disease. High frequencies of the enzyme deficiency are found only in the malarious regions. On theoretical grounds it might also be expected that the enzyme-deficiency trait might limit multiplication of malaria parasites. *Plasmodia* require reduced glutathione for growth *in vitro*[26] and about 50 per cent of the glutathione of the red cell contributes to the cysteine requirements of the parasites.[27] Enzyme-deficient cells have a subnormal concentration of reduced glutathione, which in a variety of conditions is depleted to very low levels.[21] Moreover, there is evidence that malaria parasites use the hexose monophosphate shunt pathway in red cells,[28] and that the rate of metabolism by this pathway is reduced in enzyme-deficient cells. Hence for at least two reasons enzyme-deficient cells would be unlikely to support maximal growth of malaria parasites.

A direct field test of the malaria-protection hypothesis was carried out in collaboration by Allison and Clyde[29] in Tanganyika. Male children with enzyme deficiency between four months and four years of age were found to have significantly lower parasite counts than children with normal enzyme activities. Female children with low enzyme activities (mostly heterozygotes) had fewer parasites than those with normal enzyme activities although the results were not statistically significant.

Through the courtesy of I. A. McGregor, enzyme estimations were made on 31 Gambian children who had been examined daily for the first two years of life for clinical signs of malaria and number of parasites. Three enzyme-deficient males were included in the sample. Although these children had occasionally high parasite counts, the peaks of high parasitemia were briefer than in most other children; the only two children in the whole group who were judged not to be in danger from malaria throughout the two-year period and who received no antimalarial therapy, were enzyme-deficient. Although these numbers are small, they represent a unique sample that was very carefully studied, and the results are consistent with the hypothesis that the enzyme-deficiency trait affords protection against malaria.

Motulsky,[24] working with Vandepitte on Congolese children chiefly between 5 and 10 years old, was unable to demonstrate any effect of the enzyme deficiency on malaria parasite rates, but it might be expected that in this age group any protection would be overshadowed by the effects of acquired immunity.

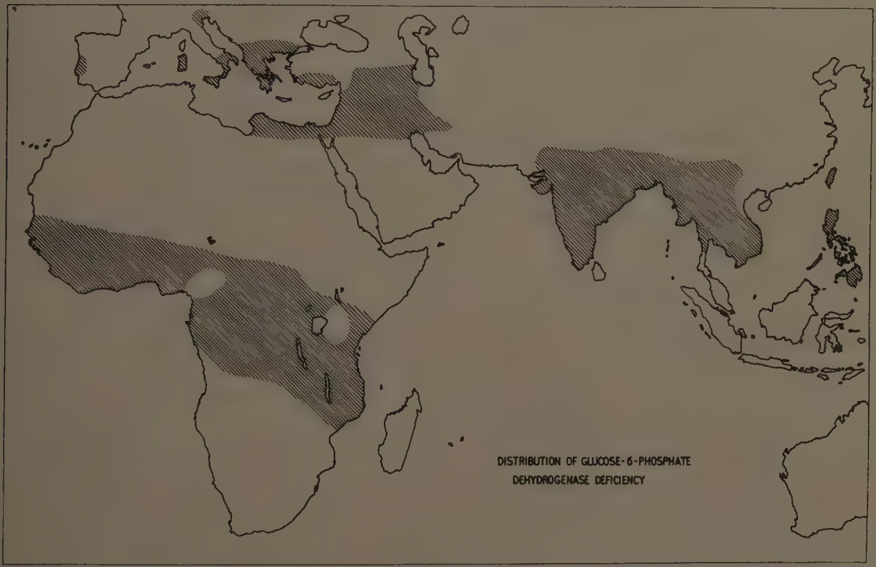
DISTRIBUTION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

FIGURE 5. Known distribution of the glucose-6-phosphate dehydrogenase-deficiency gene in frequencies above 0.02 in the Old World (data of Motulsky[24] and Allison[25]).

Selection in Populations Exposed to Malaria

Because of selective mortality from malaria, African children with the sickle-cell trait have a greater chance of surviving to reproductive age than children without the trait. This has been shown directly[25] by finding that in populations of infants the ratios of sickle-cell heterozygotes and homozygotes to normal subjects are in close agreement with those expected from the random-mating hypothesis, whereas in the same population at reproductive age there is a significant excess of sickle-cell heterozygotes. Similar observations have been presented by Vandepitte and Delaisse.[12] J. and C. Lambotte-Legrand,[13] made an illuminating comparison of the frequency of sickle-cell trait in children dying in the pediatric clinic at Léopoldville, the Republic of the Congo, between the periods 1949–1950 and 1956–1957. In the interval between these periods holoendemic malaria was eradicated in the neighborhood of Léopoldville; in the earlier period, sickle-cell trait children contributed 15.7 per cent of deaths in the pediatric clinic, but in the later period (when only 1 malarial death was recorded) they contributed 20.5 per cent: almost equally with the normal children in the area. The change was attributed to the virtual elimination of malarial mortality, and this is the first demonstration on a demographic scale that removal of the malarial hazard can take away the genetic advantage from sickle-cell heterozygotes.

Although there is general agreement that the mortality from malaria in children with the sickle-cell trait is lower than in other children, a second factor deserves consideration, as Livingstone[31] in particular has emphasized. This is the possibility that greater resistance to malaria may result in increased fertility of sickle-cell trait carriers as compared with nonsicklers. Two possible ways in which protection against malaria might increase female fertility have been suggested. It has long been accepted that (1) malarial infection may lead to abortion; and (2) a higher frequency of premature births (defined by low birth-weight) in malarial mothers has been established for southern Nigeria.[32] If the still-birth, abortion, or neonatal death rate were thus reduced for pregnancies of women with the sickle-cell trait, this could contribute to the persistence of the sickle-cell gene in the population.

In considering these possibilities it has to be borne in mind that, as stated above, the effect of the sickle-cell trait on parasite counts in the blood of adults is negligible; nevertheless, there might be some effect on placental infection. Family studies have provided no evidence of increased fertility among sickle-cell heterozygotes.[9,33] When males and females were considered separately, there have been suggestions of increased male fertility[34] and female fertility,[32,35] although another study has shown no increase in female fertility.[36] An indirect approach to the problem is by estimating the sickling frequency in antenatal cases, but here again the findings are contradictory: Jacob[37] reported a lower frequency of sicklers among expectant mothers in Uganda, and Garlick[15] a higher frequency among expectant mothers in Nigeria, than in the corresponding general populations. Garlick's genetic analysis of the mother-and-child pairs suggested that increased female fertility might occur in sickle-cell heterozygotes, although the fit between the observed values and those predicted by the differential viability hypothesis is satisfactory, so that there is no need to invoke differential fertility to explain the results.

In summary: there is substantial evidence that children heterozygous for the sickle-cell gene have a greater chance of surviving between birth and reproductive age than other children. This would appear to be the chief selective effect of malaria. It is also possible, but not proved, that some selection is achieved through differential fertility of women.

Genetic Response of Populations to Challenge by Malaria

Available evidence[38] supports the view that would be expected on general grounds: that the sickle-cell gene is a rare mutant. It might have arisen independently in the several areas where the gene is now common (Africa, Sicily, Greece, south Turkey, India, and Assam). It can be concluded that in malarial regions the frequency of the gene increased until the advantage of the heterozygote was counterbalanced by the disadvantage of the homozygote, and a stable equilibrium maintained thereafter while malaria persisted. The same was probably true of the glucose-6-phosphate dehydrogenase-deficiency gene although, in this case, the sex linkage makes the population genetics more complex. The female heterozygote is probably resistant to some extent against malaria, and so is at a selective advantage whereas the male hemizygote—being liable to hemolysis from virus disease and perhaps other causes—may actually be at a net disadvantage. This is the simplest way in which a stable equilibrium at a sex-linked locus could be maintained.[39] If the abnormal homozygote and hemizygote were at a net advantage, the enzyme-deficiency gene might have replaced the normal allele in some populations, whereas the frequency of the former is always less than 0.4.

Both the sickle-cell and enzyme-deficiency genes attain high frequencies only where malaria is (or was until recently) hyperendemic or holoendemic. There is a clear positive correlation between the frequencies of the two genes in populations (FIGURE 5), which is to be expected if they are not disadvantageous in combination. A similar positive correlation between the frequencies of the thalassemia and enzyme-deficiency genes in Sardinia has been found by Motulsky[24] and Siniscalco. This is in contrast to the situation in west Africa where both the sickle-cell and hemoglobin C genes are found in the same populations. In this case individuals inheriting both abnormal genes have a variant of sickle-cell disease and are at a disadvantage. For this reason Allison[40] postulated that the two genes should tend to be mutually exclusive in populations. This was subsequently found to be the case (FIGURE 6) in most west African tribes. Hemoglobin C has a distribution restricted to west Africa, the incidence of heterozygotes rising to 29 per cent in northern Ghana, and as the frequency of the hemoglobin C rises that of the sickle-cell gene falls.[9,41] Some populations, including Liberians, have low frequencies of both the sickle-cell and hemoglobin C genes. The reasons why this should be so are far from clear, but one factor could be the relatively high frequency of carriers of the typical thalassemia gene in Liberia.[42] Since the sickle-cell and hemoglobin C genes are known to be disadvantageous in combination with thalassemia, it would be difficult for the former two to attain high frequencies in populations where thalassemia is already established.

Theoretical genetic calculations[30] indicate that the hemoglobin C heterozygotes should be at an advantage in west Africa. The only investigation so far carried out on rather small numbers[43] has not shown this genotype to have resistance to *falciparum* malaria. For equilibrium a much smaller advantage than that of the sickle-cell heterozygote would suffice, and it would be difficult to obtain unequivocal evidence of its existence in field studies. The distribution of this trait in west Africa suggests that protection against other species of malaria parasites may occur (Livingstone, unpublished), and further investigations of this problem would be worth while.

An even greater need exists for further work on other abnormal hemoglobins and malaria. The thalassemia gene is as lethal as the sickle-cell gene when homozygous, and yet heterozygote frequencies up to 20 per cent have persisted in parts of Italy, Greece, and Asia (FIGURE 9). The heterozygote must therefore be (or have been until recently) at a considerable disadvantage, and once again the local distribution in countries such as Sardinia,[44] as well as the overall distribution in world populations, strongly suggests that *falciparum* malaria is involved. In several southeast Asian countries, such as Burma, Thailand, and Indonesia, both the thalassemia and hemoglobin E genes are common (FIGURE 10). The latter is probably disadvantageous only rarely when homozygous, but there is no denying the disadvantage of individuals who inherit both the hemoglobin E and thalassemia genes. The only plausible inference is that the possession of a single abnormal gene is advantageous, and the distribution of the character again brings malaria to mind. The indirect evidence suggesting that thalassemia (and possibly hemoglobin C and E) heterozygotes are resistant to malaria is of interest in relation to the mechanism by which the resistance might be achieved. Thalassemia heterozygotes often show only slight hypochromia, microcytosis, ovalocytosis, basophilic stippling, and low but definite fetal hemoglobin concentrations. It is remarkable that such trivial

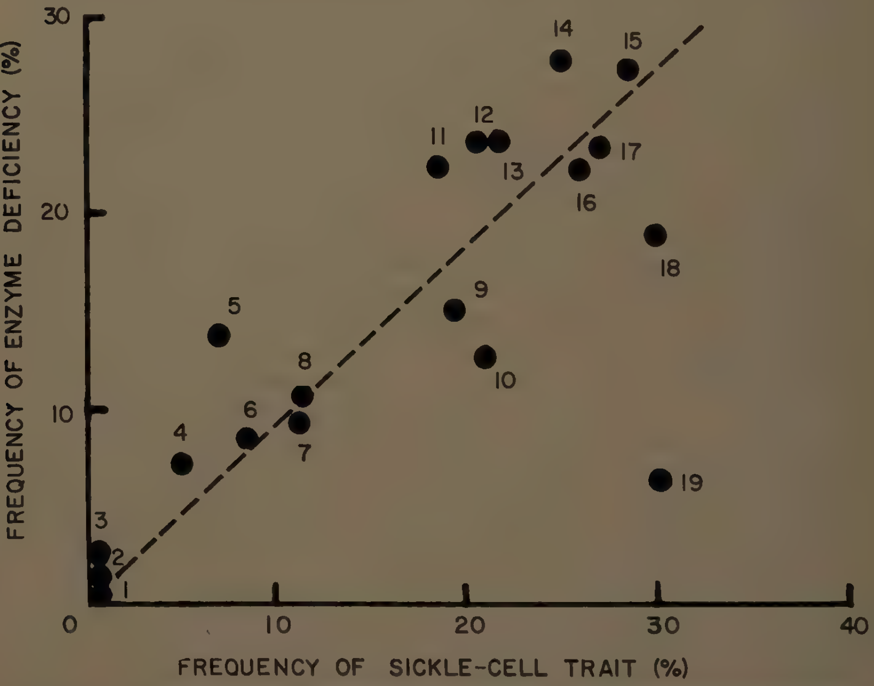

FIGURE 6. Frequencies of the sickle-cell and glucose-6-phosphate dehydrogenase-deficiency traits in African populations. Combined data of Motulsky[24] and Allison.[25] Key: (1) Watutsi; (2) Masai and Kikuyu; (3) South African Bantu; (4) Usumbura; (5) Bashi; (6) Bahutu; (7) Bwaka; (8) United States Negro; (9) Buganda; (10) southern Nigerians; (11) Samba; (12) Zigua; (13) Bayaka; (14) Luo; (15) Bondei; (16) Léopoldville Africans; (17) Digo; (18) Stanleyville Africans; and (19) Pygmies. There is reason to believe that the enzyme-deficiency rate given for Pygmies is too low (Motulsky, private communication).

changes should affect the multiplication of malaria parasites. However, the relationship between the parasites and host cells may be extremely delicate, so that small changes in the host cells could limit the growth or division of parasites. Most species of *Plasmodium* appear to be very sensitive to their cellular environment, as shown by their host specificity; this is manifested even on intravenous injection, so that the exoerythrocytic phase is not involved. Thus when heavy *P. falciparum* trophozoite inoculations were given to monkeys, only slight and transient parasitemias were observed,[45] and the same was true when *P. knowlesi* was given to man.[46]

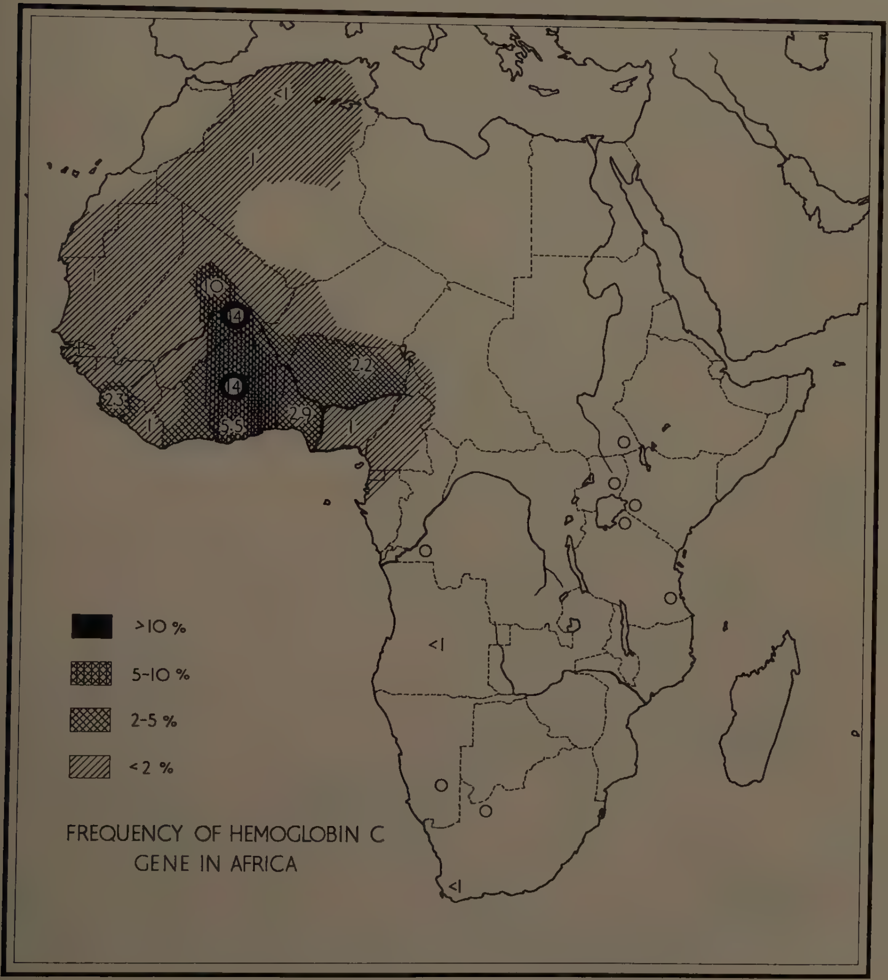

FIGURE 7. Distribution of hemoglobin C gene.

If the thalassemia heterozygote is resistant to malaria, it follows that erythrocytes in iron-deficiency anemia, showing similar changes, might also be un-

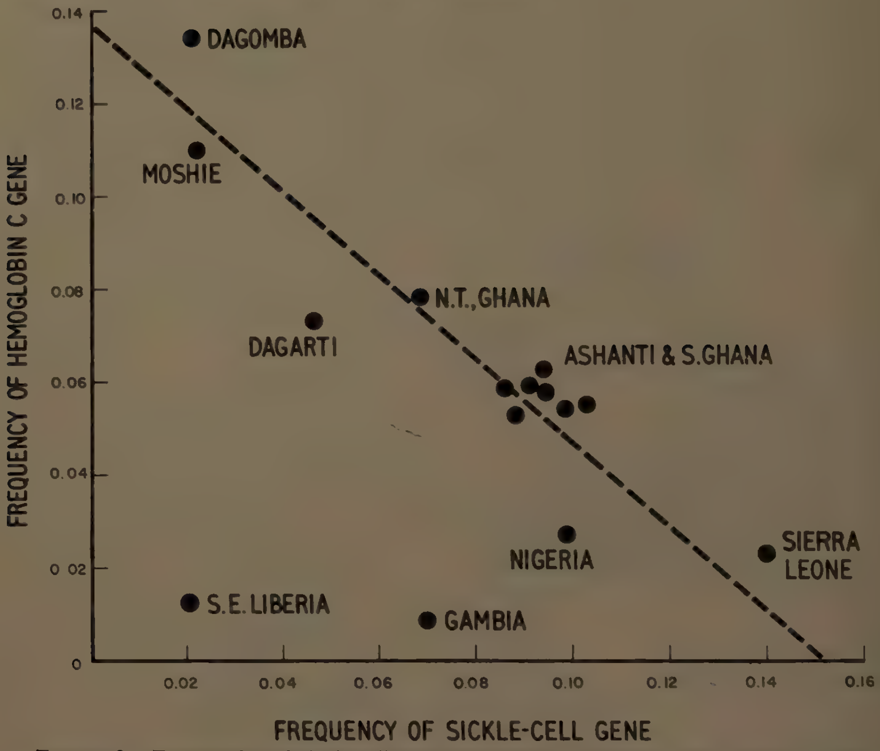

FIGURE 8. Frequencies of sickle-cell and hemoglobin C genes in west African populations.

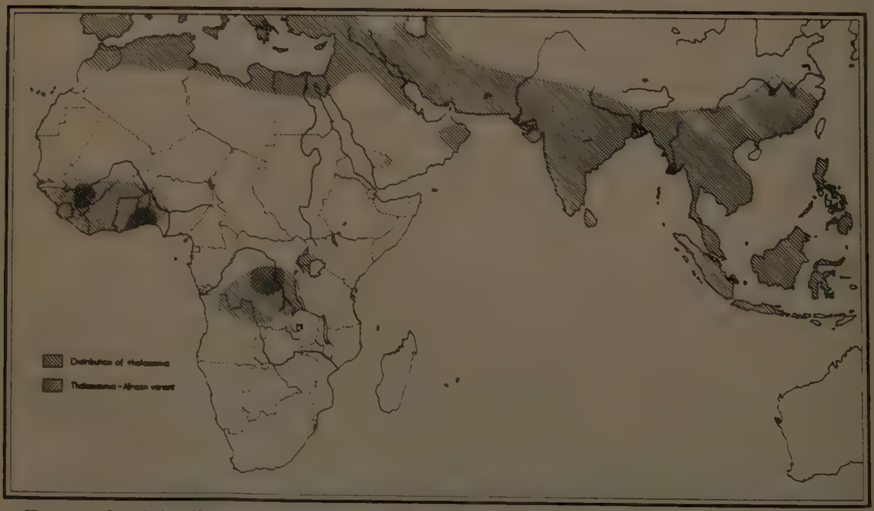

FIGURE 9. Distribution of thalassemia and the African variant (persistent fetal hemoglobin gene).

favorable for growth of parasites. This leads naturally into the effects of nutrition on the progress of the disease, which will be considered briefly below.

Summation of Inherited and Acquired Immunity to Malaria

Attention has already been drawn to the fact that the differences in malaria parasite rates and counts between sickling and nonsickling children are most

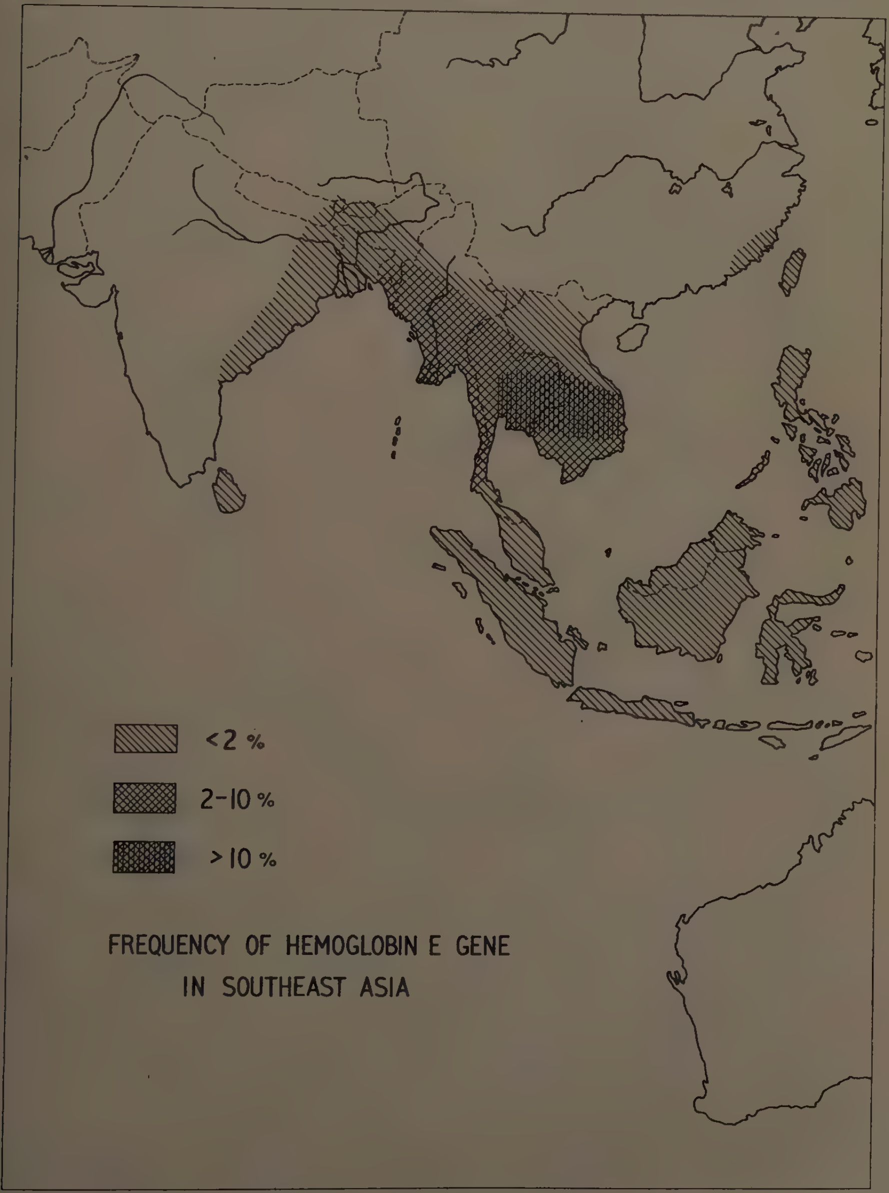

FIGURE 10. Frequency of the hemoglobin E gene in southeast Asia.

pronounced not in infants first exposed to malaria, but in the one- to four-year age group. This has been a consistent finding in many studies from east, west, and central Africa. Hence it would appear that acquired immunity actually reinforces the protection afforded by the inherited hemoglobin factor during the intermediate phase before the former becomes fully effective. This result is not unexpected, since young sickling children certainly have parasites for long periods, and there is no reason to suppose that their lower parasite numbers are less efficient in generating immunity than the higher parasite numbers in nonsickling children. Indeed, it is conceivable that in sickling children a higher proportion of parasites reach the spleen or other antibody-producing organs, or that the briefer infections in the intermediate age group allow more rapid reinfection with other strains of *P. falciparum*, so that immunity to a large variety of strains is built up quickly. Whatever the explanation, it has been found that sickling children from Nigeria have higher γ-globulin levels than nonsickling children,[47] which is consistent with the interpretation that the presence of the sickling trait actually enhances the antibody response against the malaria parasite.

Summation of natural and acquired resistance to malaria is also apparent in other situations. Thus Milam and Coggeshall[46] challenged human subjects with homologous parasites after a single cycle of growth of *P. knowlesi*; all were found to be completely immune. In contrast, monkeys are susceptible to reinfection with homologous parasites when they have been cured of their chronic *P. knowlesi* infections.[48]

Additive effects of hemoglobin resistance and acquired immunity might also explain the apparent discrepancy between the reports of Allison[10] and of Beutler *et al.*[50] on results of experimental inoculation of *P. falciparum* into human subjects with and without the sickle-cell trait. In Allison's study the subjects would have had appreciable immunity, and only a minority of sickle-cell trait carriers showed parasites. In the study of Beutler *et al.* the subjects were nonimmune, and all showed parasites, the parasite counts in sickle-cell trait subjects being, however, significantly lower than in the others. A comparable field situation where partial immunity probably reinforced the hemoglobin effect has been described by Deliyannis and Tavlarakis.[51] Greek children (many of school age) with the sickle-cell trait were found to have much lower parasite rates and counts than those without the trait.

Other Genetic Factors in Immunity to Malaria in Man and Experimental Animals

There is little doubt that the possession of abnormal hemoglobin and glucose-6-phosphate dehydrogenase deficiency traits is not the only reason why Africans are more resistant to malaria than Europeans. Only a minority of United States Negroes have these traits; yet it is generally agreed that nearly all United States Negroes show after inoculation considerable resistance against several species of malaria parasites, including *P. vivax*[49] and *P. knowlesi*.[46] The difference in susceptibility of United States Negroes and whites—who have had no previous exposure to the disease—suggests that other factors, probably under complex or polygenic control, are involved as well as the major gene effects already mentioned. It is obviously very difficult or impossible to analyze factors in man, but useful studies could be carried out on experimental animals.

The most suitable animal for such studies is the mouse, since highly inbred strains are available showing considerable differences in susceptibility to *P. berghei.* Useful work on individual strains and hybrid offspring has already been published by Greenberg and Kendrick.[52] Unfortunately their conclusions are not definitive since they failed to take into consideration a factor that may again be of importance, namely, hemoglobin types. Several investigations have shown that hemoglobins differ in different strains of mice; the best resolution has been achieved by starch gel electrophoresis, which shows five hemoglobin components in some strains.[53] The mouse hemoglobins differ also in solubility,[54] and this might well affect the growth of parasites. Preliminary observations, made in collaboration with B. S. Blumberg of the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md., have shown that strains Streak (which is highly resistant to *P. berghei*) and Swiss (which is highly susceptible) have hemoglobin types different from one another and from other strains of intermediate susceptibility to experimental malaria. Whether the apparent association is fortuitous or meaningful can be ascertained only by following segregation of the various factors in second-generation hybrids. If mouse hemoglobin types prove to be related to resistance against malaria, an excellent model system for analysis of the interaction of single-factor and multi-factor resistance with one another and with environmental factors (such as nutrition) will be available. If there is no association with hemoglobin type, the system would be valuable as an example of multi-factor control of resistance against a disease.

The Role of Host Nutrition in Malaria Resistance

Although detailed discussion of environmental factors is beyond the scope of this paper, the opportunity is taken to emphasize that the genetic factors that have been considered manifest themselves in particular environmental situations that may have an important bearing on the outcome. Thus it is exceptional for an African child in the malaria-susceptible age group to have a hemoglobin concentration that is normal by western European standards. As mentioned above, this might conceivably limit the multiplication of parasites and so reinforce protective affects of inherited traits and acquired immunity.

A number of nutritional deficiencies in host animals have been reported to limit multiplication of malaria parasites. These include depression of *P. lophurae* in riboflavin-deficient and vitamin A-deficient chicks,[55,56] depression of *P. gallinaceum* in pantothenic acid-deficient chicks,[57] depression of *P. berghei* in *p*-aminobenzoic acid-deficient mice,[58] and of *P. knowlesi* in ascorbic acid-deficient and starving monkeys.[59,60] However, the effects are complicated by the fact that nutritional deficiency might also lower the antibody-producing capacity and general resistance of the host. This is apparent from observations that deficient animals sometimes succumb from infection even though parasite counts are low.[55] Furthermore other deficiencies, such as biotin and nicotinamide deficiencies in chicks, actually result in increased malaria parasite counts.[55,56]

Other factors—such as simultaneous exposure to other diseases—complicate the natural picture of malaria in the field still further. In view of this complexity it is remarkable that it has been possible to demonstrate so consistently the effects of the sickle-cell and glucose-6-phosphate dehydrogenase-deficiency traits in the right age groups. Although further field work is required in relation to thalassemia and other abnormal hemoglobins, it is clear that this type of work is becoming increasingly difficult to carry out for political and other reasons. The principles of single-gene resistance and natural selection in response to disease have been firmly established in man, and the time has come to make fuller use of model experimental systems such as that described above, analysis of *P. berghei* in mice.

Multiplicity of Genetic Responses to the Challenge of Disease

The question arises whether the resistance afforded by the sickle-cell trait against malaria is an isolated, chance happening or "evolutionary fluke"—as Neel[61] has suggested—or whether it is an example of a whole class of genetic responses to disease. I have no doubt that the latter is true, that there exists a large series of mutations affecting the structure of proteins and glycoproteins that provide resistance against particular diseases or unfavorable circumstances. These are quite apart from proteins involved in classical immunological responses. Such mutations would give rise to organisms at a selective advantage when the disease is prevalent, and the mutants would increase in frequency in exposed populations. This would appear to be a mechanism of resistance very widespread among living organisms. Thus, a staphylococcus exposed to penicillin produces a protein, penicillinase, which allows the staphylococcus to proliferate in the presence of the drug; the capacity to produce this enzyme is the result of one mutation. Single-gene mutations in many bacteria confer resistance against particular bacteriophages. Wallace, elsewhere in this monograph, recalls how among plants many examples of resistance against disease are known to be controlled by single genes, and the biochemical basis of this resistance is presumably to be found in altered protein molecules. In flies, resistance against high doses of DDT is conferred by a single gene, which controls the formation of a specific enzyme, dehydrochlorinase, responsible for inactivation of the insecticide.[64]

This, then, would appear to be the simplest and most widespread type of resistance. There are obvious analogies with the sickle-cell situation, which is, in any case, not an isolated response to disease since a similar situation obtains in the case of the glucose-6-phosphate dehydrogenase-deficiency gene. The evidence that certain diseases fall unequally upon possessors of different blood-group genes[62] provides several other examples. The diseases studied can hardly be assumed responsible for much selection in human populations or to have led to the blood-group polymorphisms now observed. Other diseases, in particular bacterial infections of childhood that have produced a considerable mortality in most populations, deserve closer study. It is known that certain bacteria have antigens structurally similar to blood-group substances. Hence the possession of particular blood-group antigens might affect resistance or susceptibility to given bacteria in various ways. To take two simple mechanisms, it is conceivable that the presence of naturally occurring isoagglutinins like anti-A or anti-B might confer resistance, or that antibodies to bacterial antigens similar to those of the host would not be formed owing to immunological tolerance, thus increasing susceptibility. There are large areas in the world today where these diseases still produce considerable childhood mortality, and investigation of their relationship to blood groups would seem to be most worth while.

In general it can be supposed that in vertebrates there is superimposed upon such major-gene systems of resistance a whole series of immune responses. These are complex, involving both cellular and humoral types, and including in the latter the numerous components of complement. With the emergence of the clonal selection theory of antibody production in its complete form[65] the distinction between this mechanism and single-gene resistance may not be as great as was formerly supposed.

A third class of host responses to disease is provided by tissue inhibitors of virus growth, in particular, interferon, which appears to play a part in the recovery from virus infections before antibody is demonstrable.[63] This may well turn out to be the utilization of enzymes normally present in cells for production of a protein-limiting virus growth, and again may be a very general and widespread mechanism of resistance against disease.

In bacteria yet another mechanism exists: this is the state of lysogeny in which viruses are incorporated into the chromosomal structure of the host cells and provide resistance against superinfection with related, virulent viruses. No convincing evidence has been forthcoming that any such situation obtains in higher organisms, but when cell genetics are better understood more critical tests for its presence or absence can be made.

Even without lysogeny the responses of host organisms to the challenge of disease are evidently very complex. Many possible mechanisms of resistance, under more or less direct genetical control, can come into play and continue to produce organisms that can multiply in the presence of the challenge. They can therefore allow survival of the host population even when the agent producing the disease is itself changing.

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GENETIC DETERMINANTS OF COMMUNICABLE DISEASE*

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The purpose of this paper is to review my experience in an attempt to clarify the part played by genetic differences in the occurrence of communicable diseases in man, to assess successes and limitations, and to forecast, although with some trepidation, the direction of future efforts.

The concept that genetic differences may be an important factor in the occurrence of these diseases cannot be regarded as something new. It has played a part in the thinking of clinicians and epidemiologists since the time of Hippocrates at least. Of interest for this present discussion are the changes in concept that have occurred during the past 75 years. Discovery of the role of specific agents in communicable diseases resulted in the development of treatment and preventive measures of great importance for human society. Desire for the widest acceptance and application of these measures led to an emphasis on the role of agents and found its most extreme expression in the doctrine of single causes. During this period students of human heredity were engaged in more general studies, and their efforts did not meet with comparable rewards in treatment and prevention. It is quite understandable, therefore, that the idea of a single cause—identified as the agent—became widely accepted. Experience soon demonstrated that factors in the environment and, indeed, in the host too, played indispensable roles. Although some adjustments were introduced to account for the results of later studies, the single-cause doctrine retained its fundamental appeal. To some extent, geneticists helped to maintain this view beyond its useful period by their willingness to debate the highly polarized challenge: nature or nurture.

More recently we have begun to see the return to a more balanced view. Today epidemiologists regard human disease as resulting from the interaction of agent, host, and environmental factors. The principles of disease causation have been well summarized in a recent paper by Clark (1955). Disease and disorder, he says, "are the result of an imbalance between agents of disease and man; the extent of this imbalance depends upon the characteristics of the agents and those of man; and these characteristics are largely governed by the conditions of the environment. Thus, within the agent, the host and the environment are all the conditions and circumstances that initiate and perpetuate a disease process. The process actually begins before man himself is involved. There are genetic and environmental influences which determine whether or not man will come in contact with disease agents (infectious or noninfectious) and once man and agent meet, similar influences may determine the outcome of the interaction. There are two related processes, one taking place in the environment before man is actually affected and the other, the process of interaction of man and disease agent (the course of the disease in man)."

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There is one additional aspect of epidemiology that deserves mention. For accurate description of disease, observations should not be limited to the sick (which, of course, is the primary concern of clinical medicine). For community studies the unit of interest is the group, or population; description and analysis includes the well and the sick, the unaffected as well as the affected. Since this focus of attention is shared by many, no further elaboration of this point seems necessary. Recognition of these principles and their acceptance has resulted from their successful application to the solution of epidemics and their helpfulness in the mass study of both communicable and other diseases.

My reasons for starting this discussion with a somewhat theoretical statement of epidemiologic principles are two. First, disease occurrence, the end point of our observations, is the outcome of the process I have described: that is, the interaction of disease agents and the human host within a specified environment. The number of reactions that intervene between genetic determinants in the cell nucleus and the expression of disease is unknown, and may involve the activity of other genes as well as the influence of environmental factors. Accordingly, information derived from studies of disease may not give a key to the activity of determinants. Development of laboratory procedures for the study of the chemistry of heredity may one day free us from this limitation, but this is for the future.

My second reason for recalling basic principles is that interpretations of studies, particularly practical application for treatment and prevention of disease, are simplified when considered in terms of interaction. Although in any given situation the imbalance that results in communicable disease may be due to a genetic change in the host, re-establishment of the balance in favor of man may be brought about by a variety of measures, such as reducing the prevalence of the agent, modifying the susceptibility of the host by diet or immunization, or altering the physical, biological, or social environment. Osborne and DeGeorge (1959) have recently emphasized the futility of attempting to institute the controlled breeding of man for this purpose.

From these brief comments on epidemiology, I shall proceed directly to a consideration of data bearing on the role of genetic differences in the occurrence of communicable disease in man. The preceding papers in this monograph provide the experimental background for my remarks. To use a figure of speech: others before me have stumbled over loose boards in trying to recarpenter model houses (based on experiments with animals) into frame dwellings to accommodate human disease. Perhaps I shall fare as badly, but I consider that Webster's studies (1937), showing the importance of genetic differences in animal hosts, and Schneider's (1956) observations on the role of nutrients in modifying genetic susceptibility to infectious agents will find applications in the study of human disease. In this monograph Gowen and Hutt give illustrations from their extensive studies. Allison's paper gives me the opportunity to forewarn you that the data I present are, in many respects, far less complete than he has been able to present.

If I enumerated all the communicable diseases for which the claim of genetic determinants in man have been advanced, the list would be quite large. However, in most cases the supporting data are meager and, in large part, not acceptable according to modern standards for genetic and epidemiologic studies. For this discussion therefore, I have drawn on the literature for only three diseases: poliomyelitis, tuberculosis, and rheumatic fever. Although rheumatic fever is not a communicable disease in the ordinary sense, I have included it to illustrate techniques for the study of interaction of genetic and nongenetic factors. For each of the three diseases, pertinent studies are examined. After presenting the results, I then discuss the values and limitations of various types of studies that have been used in exploring the role of genetic determinants.

Poliomyelitis

In 1942, Aycock summarized the results of observations made over a period of 17 years. Starting from the clinical impression that members of the same family were attacked by paralytic poliomyelitis in succeeding outbreaks, Aycock assembled an impressive array of data from his own and other studies. He found that the secondary attack rate among exposed siblings was considerably higher than among exposed unrelated individuals. He reported recurrences in different years among siblings (in 22 families), among parent and offspring (in 38 families), and among other relatives; in many cases the disease occurred in individuals not yet born when the primary case in the family occurred, or after the primary case was no longer in the household. Data were also presented concerning a dozen small rural outbreaks where family concentration was pronounced.

Perhaps most important was Aycock's (1942) comparison of families of 586 poliomyelitis patients in Massachusetts and Vermont with a series of 100 controls. Aycock was interested in the occurrence of the disease in relatives of patients other than members of the same household; he found the disease in 26.8 per cent of the poliomyelitis families and in only 5 per cent of the controls (TABLE 1). He recognized that these data were not part of a preplanned investigation and cautiously suggested that the familial aggregations "... are enough to warrant the inclusion of similar studies in systematic epidemiologic investigations."

This promising lead was soon followed by other investigations. In the same year, Addair and Snyder (1942) collected all cases of paralytic poliomyelitis that could be located in an isolated section of West Virginia. There were 29 cases; these had occurred during a period of 50 years, and all of the individuals involved were found to be related to each other. Working along similar lines, Reedy (1957) made inquiries among the families of poliomyelitis cases that had occurred during the 1950 outbreak in the vicinity of South Bend, Ind. Among 50 families living within a radius of 100 miles, information was collected on 5677 family members. As in Aycock's study, reported cases were verified by a review of epidemiologic and hospital records and by correspondence with physicians. The frequency of additional cases among relatives was 6.75 per 1000, compared with less than 2 per 1000 in the general population of the area.

In order to explore further the possible role of genetic determinants in susceptibility to paralytic poliomyelitis, Herndon and Jennings used as their primary source reports of cases filed by the attending physicians with the Division of Epidemiology of the North Carolina State Board of Health. These reports are required by law to be filed in each case where poliomyelitis is diagnosed. Inquiry was made regarding all cases reported from 1940 to 1948. Through physicians and other sources, a total of 46 families were located containing twins or triplets, in which one or more of the twins or triplets had had poliomyelitis while living with the twin partner (TABLE 2). All twins were examined in order to supplement previous medical and hospital reports.

With regard to paralytic poliomyelitis, concordance in monozygotic and dizygotic twins was 35.7 per cent and 6.1 per cent respectively; this difference is statistically significant at the 1 per cent level. Herndon and Jennings (1951) concluded from their investigations that there is, at least in part, a genetic factor controlling susceptibility to the paralytic form of poliomyelitis.

TABLE 1

OCCURRENCE OF POLIOMYELITIS IN RELATIVES OF PATIENTS AND CONTROLS

	Total	Cases*	
		Number	Per cent
Relatives of patients	586	157	26.8
Relatives of controls	100	5	5.0

* Exclusive of cases in household of index.
Data from Aycock, 1942, p. 456.

TABLE 2

OCCURRENCE OF PARALYTIC POLIOMYELITIS IN CO-TWINS

	Total examined	Paralytic poliomyelitis	
		Cases	Per cent
Dizygotic co-twin	33	2	6.1
Monozygotic co-twin	14	5	35.7 P = 0.009

Data from Herndon and Jennings, 1951, p. 43.

Addair and Snyder, as well as Reedy, suggested recessive inheritance with penetrance of about 70 per cent. No conclusions concerning the genetic mechanism were drawn by Aycock or by Herndon and Jennings. However, the latter suggested that their data were compatible with the hypothesis of a recessive gene with a very much lower penetrance, that is, about 35 per cent.

Tuberculosis

Prior to the discovery of the tubercle bacillus in 1882, the idea of heredity in tuberculosis was widely accepted. This view may be epitomized by a statement of Hirsch, one of the forerunners of modern epidemiology, who wrote in 1883, ". . . the severest sceptic can hardly venture to deny a hereditary element in this case." Clinical impressions and other observations on which this conclusion was based would hardly be acceptable today for the following reasons: (1) the use of X-ray examinations and more information on the natural history (particularly on the clinical course of tuberculosis) permit more satisfactory diagnosis and clinical classification; (2) we no longer accept the concept that transmission of an infection (for example, syphilis) from mother to child is within the scope of heredity, which is defined as the character and behavior of genetic determinants; and (3) we differentiate between natural and acquired immunity, or insusceptibility. For these reasons I do not include a review of the earlier literature. I should be remiss, however, if I did not acknowledge that the quotation I have cited above was taken out of context and for that reason is a distortion of Hirsch's broad characterization. For those who are further interested, may I suggest that the monograph of Puffer (1944) includes a readable and more sympathetic review of earlier studies.

Concentration of tuberculosis in families and its varying prevalence in geographic areas and in ethnic and other identified groups is well known. For tuberculosis, these differences in themselves cannot be considered as proof of the presence of genetic determinants since the important role of exposure has been demonstrated. It is also known that familial concentration is commonly observed in other communicable diseases as well as in parasitic and dietary diseases. Accordingly, in the search for differences in host susceptibility, twin studies and long-term epidemiologic studies of familial aggregation should be considered. For discussion I have selected studies conducted at the New York State Psychiatric Institute, New York, N.Y., and in Williamson County, Tenn.

TABLE 3
TUBERCULOSIS IN 308 TWIN INDEX-FAMILIES

Relationship to twin index	Number observed	Tuberculosis	
		Cases	Per cent
General population (estimate—New York State)			1.1
Spouse	226	14	6.2
Half-sibling	42	4	9.5
Parent	688	114	16.6
Sibling	720	136	18.9
Dizygotic co-twin	230	42	18.3
Monozygotic co-twin	78	48	61.5

Data from Kallman and Reisner, 1943, TABLE 6.

Kallman and Reisner (1943) assembled a series of 657 twins with diagnosed tuberculosis from tuberculosis hospitals and clinics in the state of New York. All co-twins were interviewed and examined, in addition to other family members whenever possible, to supplement hospital and clinic records. In the published report, data were presented regarding 230 dizygotic and 78 monozygotic pairs and members of their families.

Occurrence of tuberculosis in members of these families over the age of 14 years was considerably higher than in the general population, and increased in proportion to the degree of blood relationship to the index person (TABLE 3). The morbidity rate among parents, siblings, and dizygotic co-twins was about equal: 16 to 19 per cent. It was lower for half-siblings and spouses, and highest for monozygotic co-twins: 61.5 per cent. In comparison, the adjusted morbidity estimate for the state of New York was 1.1 per cent.

Striking differences between dizygotic and monozygotic twins were shown by Kallman and Reisner in two other comparisons: (1) among dizygotic twins known to have been exposed within the family, the prevalence was twice as high as among dizygotic twins without known exposure: 26.3 per cent compared with 12.7 per cent; among monozygotic twins, the increase was less marked: 69.2 per cent in the exposed group compared with 61.5 per cent in the group without known exposure (TABLE 4); and (2) when tuberculosis was present in one or both parents, the morbidity rate among siblings and dizygotic co-twins was twice as high as in families where both parents were nontuberculous: about 37 per cent compared with 18 per cent. Differences among monozygotic twins were minor (TABLE 5).

TABLE 4

TUBERCULOSIS AMONG CO-TWINS IN RELATION TO EXPOSURE WITHIN THE FAMILY

Exposure in family	Dizygotic co-twins			Monozygotic co-twins		
	Number examined	Tuberculosis		Number examined	Tuberculosis	
		Cases	Per cen		Cases	Per cent
Known	175	46	26.3	52	36	69.2
Not known	55	7	12.7	26	16	61.5

Data from Kallman and Reisner, 1943, TABLE 11.

TABLE 5

ASSOCIATION OF TUBERCULOSIS IN PARENT AND OFFSPRING

	Morbidity rate*		
	Siblings (%)	Dizygotic co-twins (%)	Monozygotic co-twins (%)
Both parents nontuberculous	18.1	18.8	85.7
One or both parents tuberculous	35.3	38.6	88.9

* Age—adjusted.
Data from Kallman and Reisner, 1943, TABLE 8.

In a later paper from the same laboratory, Planansky and Allen (1953) compared the clinical course and outcome in monozygotic and dizygotic twin pairs, both members having tuberculosis, after enough time had elapsed so that the clinical course was judged to be complete in the majority of cases. These investigators reported that the clinical course and outcome were as varied in monozygotic as in dizygotic twin pairs. As seen in TABLE 6, the outcome in monozygotic twins is less often similar than in dizygotic pairs, although the difference is not significant. With regard to the initial stage, onset within

1 year was more frequent in monozygotic than in dizygotic twins, and the character and progression of initial lesions showed greater intrapair similarity.

Two principal conclusions were drawn by the authors of these studies:

(1) "Resistance to tuberculosis is modified by a heredo-constitutional mechanism which seems to be multifactorial in its genetic nature." Although morbidity rates are modified by a variety of factors, among which they cited age, sex, race, time of observation, exposure, and diagnosis of parents, these factors are judged to be secondary, and ". . . certainly not to an extent which would explain the marked difference between the two types of twins" (Kallman and Reisner, 1943).

(2) Clinical course and outcome have relatively little dependence upon genetic factors.

TABLE 6

SIMILARITY OF OUTCOME OF TUBERCULOSIS IN TWINS

	Similar outcome	Dissimilar outcome	Total
Dizygotic twins	18	12	30
Monozygotic twins	20	22	42 $P > 0.05$

Data from Planansky and Allen, 1953, TABLE 8.

TABLE 7

TUBERCULOSIS AMONG SIBLINGS OF CASES AND CONTROLS

	Families	Siblings examined	Tuberculosis	
			Cases	Per cent
Siblings of index cases	457	2472	231	9.3
Siblings of controls (spouses of known cases)	221	1327	73	5.5

Data from Puffer, 1944, TABLE 8.

Quite different in design was the Williamson County Tuberculosis Study, which was undertaken in 1931 as a long-term clinical and epidemiologic study of tuberculosis in a specified area. The location was advantageous for this type of investigation, since individuals and families remained under observation for long periods of time and were accessible for study in the clinic and at home. In 1944, Puffer summarized the observations covering a 10-year period; the statistics were believed to be relevant for a consideration of familial susceptibility.

Data on the occurrence of tuberculosis were gathered for 2472 siblings of tuberculous index cases and for 1327 siblings of examined spouses used as controls. It was found that the disease occurred more frequently in siblings of index cases than in siblings of controls: 9.3 per cent compared with 5.5 per cent (TABLE 7). Although historical data on the occurrence of cases in members of the family were obtained, they were not judged to be suitable for analysis of the influence of exposure.

Tuberculosis death rates were consistently higher in adult siblings of index cases than in the control group. For example, in the age group 20 to 29 years, the death rate is 330.3 in the first column and 213.9 in the second column (TABLE 8). It is evident from this and TABLE 7 that siblings of persons known to have tuberculosis developed the same disease and died as a result of it more frequently than did members of the control group. The differences persisted in adult life when siblings were separated and had their own homes; perhaps exposure was different under these circumstances, but in the absence of information on the extent of exposure, Puffer (1944) concluded that "... whether or not development of tuberculosis in adult life is due to exogenous reinfection or to endogenous spread of the disease is unsettled."

TABLE 8
TUBERCULOSIS MORTALITY AMONG SIBLINGS OF CASES AND CONTROLS

Age group (years)	Tuberculosis death rate per 100,000*	
	Siblings of index cases	Siblings of controls (spouses of known cases)
Under 5	62.5	15.6
5–9	—	—
10–19	73.8	17.9
20–29	330.3	213.9
30–39	277.2	154.4
40–49	164.2	44.7
50–59	164.0	189.8
60 and over	232.6	201.8
Total	162.7	92.1

* Adjusted to age and sex of combined experience.
Data from Puffer, 1944, TABLE 9.

TABLE 9
TUBERCULOSIS IN SPOUSES IN RELATION TO EXPOSURE FROM THE MARITAL PARTNER

Diagnosis of index case	Diagnosis of spouse			
	Manifest tuberculosis (%)	Latent apical tuberculosis (%)	Latent primary tuberculosis (%)	Negative (%)
Husbands				
Sputum-positive (51)*	15.7	14.1	42.2	28.0
Other (180)	7.2	8.2	55.7	28.9
Wives				
Sputum-positive (40)	20.0	16.6	40.0	23.4
Other (134)	7.5	10.2	62.1	20.2

* Number examined.
Data from Puffer, 1944, TABLES 12 and 13.

More information was available with regard to spouses of index cases (TABLE 9). Manifest tuberculosis occurred twice as frequently in spouses living with sputum-positive index cases as it did in spouses with less exposure; for example, in husbands the rates were 15.7 per cent, compared with 7.2 per cent. For wives the difference was similar, although the rates were somewhat higher. Latent apical tuberculosis (according to the Opie system of classification) also developed more frequently in the spouses of sputum-positive index cases. About 25 per cent of exposed spouses in both series failed to give any evidence of tuberculosis. Although these observations were interpreted as leaving "... no doubt that household exposure is responsible for the development of tuberculosis," the author recognized the need for an explanation of why some of the exposed spouses failed to develop tuberculosis (Puffer, 1944).

Some insight into the role of heredity in tuberculosis may be gained by a study of spouses with different family histories. For this purpose, 66 examined spouses were classified according to whether they reported the presence of tuberculosis in a member of their immediate family, that is, a parent or sibling (TABLE 10). Manifest tuberculosis was found to be present in 33.3 per cent of spouses who gave a positive family history and in 11.1 per cent of those who did not. This was the experience with spouses of sputum-positive index cases.

TABLE 10

TUBERCULOSIS IN SPOUSES IN RELATION TO FAMILY HISTORY AND EXPOSURE

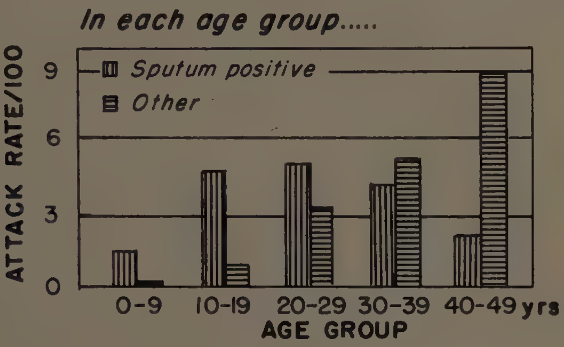
Tuberculosis in family of spouse (parent or sib)	Exposed to index case sputum positive		Exposed to index case other than sputum positive	
	Examined spouses	Manifest tuberculosis (%)	Examined spouse (%)	Manifest tuberculosis (%)
Reported	30	33.3	76	14.5
Not reported	36	11.1	93	8.6

Data from Puffer, 1944, TABLES 14 and 15.

For spouses with other exposure, the difference was small but in the same direction. The possibility that excessive childhood exposure was responsible for the higher rate seemed unlikely, since it was noted that two-thirds of the 21 persons with manifest tuberculosis and a positive family history had had no known household exposure in their childhood. The extent and activity of manifest disease was also greater in spouses with a positive family history. From these observations it was concluded that a person identified as susceptible, by evidence of the disease in parent or sibling, ran a greater risk of developing tuberculosis as the spouse of an index case than individuals not so identified. In other words, exposure to an open case increased the risk of disease among susceptible individuals.

The relation of exposure and possible familial susceptibility was examined in data for children of index cases. The percentage of children contracting tuberculosis during a given stage of life was different for children of sputum-positive parents than for other children. Children of sputum-positive parents had a high attack rate during early life; tuberculosis developed in 6.3 per cent before the 20th birthday and, in 12 per cent, 10 years later (FIGURE 1). In contrast, children of other parents (that is, other than sputum-positive) had a lower attack rate during this period: only 1 per cent were affected in the first

20 years of life and 4 per cent by the 30th birthday. In both groups tuberculosis continued to develop in adult life. Judged by total experience in the first 50 years of life, the attack rate was the same in both groups, that is, about 18 per cent in children with or without household exposure to a parent with the disease. The interpretation suggested was that children of tuberculous parents had a familial susceptibility to tuberculosis and that children exposed to an open case in early life were attacked earlier than children not so exposed.



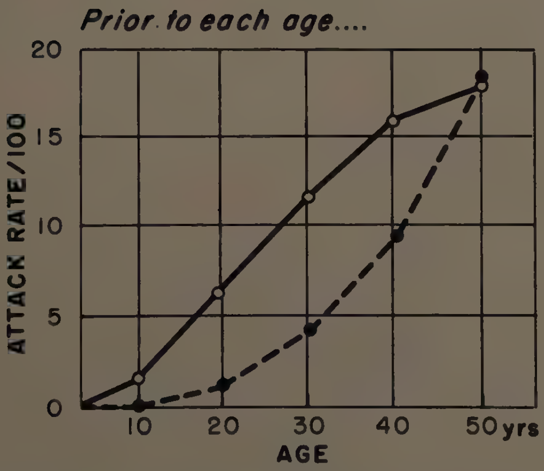


FIGURE 1. Tuberculosis attack rate among children of a tuberculous parent in relation to sputum status of parent. Data from Puffer, 1944, TABLE 20.

Data were available permitting comparison of mortality in sibships in the families of index cases where both parents, only 1 parent, or neither parent were known to have tuberculosis. Because the index case is used to identify a family as susceptible, it seemed desirable to include, for additional comparison, families that were not ascertained through an index case. For this purpose, tuberculosis mortality in sibships of spouses of index cases was used, where the parents of the spouse were known to be negative. The tuberculosis death rate among children of tuberculous parents was high, with the rate among females exceeding the rate among males in each comparison (TABLE 11). In families where both parents were known to be tuberculous, the mortality rate was high in both sexes: 400 to 500 per 100,000. The rates were lower where only 1 parent had the disease, with the rate somewhat higher where the mother rather than the father was tuberculous. Where neither parent was tuberculous, the mortality was even less, that is, below 200 in females and lower in males. In the families of spouses where neither parent was known to have tuberculosis, the mortality was below 100 in both sexes. These data are consistent with the interpretation of familial susceptibility. However, TABLE 11 is based on a rather small experience; in one half of the 10 groups there were fewer than a dozen deaths in each group.

TABLE 11
TUBERCULOSIS MORTALITY AMONG SIBLINGS IN RELATION TO FAMILY HISTORY

Family history	Tuberculosis death rates*	
	Male	Female
Parents of index		
Both tuberculous	395.1	530.6
Mother tuberculous	168.3	380.0
Father tuberculous	122.2	302.3
Neither tuberculous	66.4	175.9
Parents of spouse		
Neither tuberculous	68.0	76.4

* Per 100,000 age-adjusted to combined experience.
Data from Puffer, 1944, p. 93.

Puffer's conclusion, stated briefly, was that the evidence implicated familial susceptibility as a factor in the development of tuberculosis. The immediate risk was greatest for susceptible persons who had unusual exposure to tubercle bacilli. Interpretation of either factor, susceptibility or exposure, must include consideration of the other factor. There was no suggestion offered of a specific genetic mechanism for the postulated susceptibility.

Rheumatic Fever

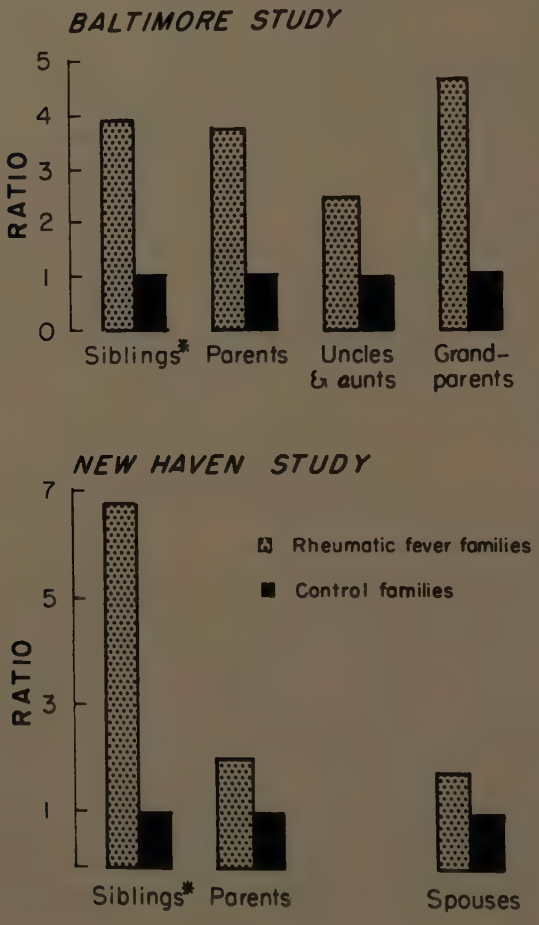
For rheumatic fever, more extensive data are available. I summarize studies from Belfast, Ireland, and from New Haven, Conn., Baltimore, Md., and New York, N. Y.

First, regarding the sources of data to be presented. The Belfast study included 462 families of children attending a rheumatic fever clinic that was opened in 1946. Supplementary visits were made to the homes during 1950 and 1951, and again 5 years later. In New Haven, 40 families, each selected through a patient known to have rheumatic fever, and 30 control families were chosen from among ward and dispensary patients who had participated in various studies from 1929 to 1939. These families had been visited and examined in the home on a number of occasions and were revisited and re-examined during 1948 and 1949. In Baltimore, family studies were initiated under the guidance of the eminent epidemiologist, Wade Hampton Frost. The index cases were 96 consecutive admissions to the pediatric cardiac clinic; 33 consecutive admissions to the pediatric tuberculosis clinic were used as controls. At New York Hospital, 112 families of patients attending the children's cardiac clinic were selected from among a larger group under close observation for many years by the same supervising physician. Many of these families have participated in studies conducted during the past 30 or more years. During the course of these studies, home visits by social workers and physicians were made at frequent intervals, and accurate observations were accumulated covering 2 and 3 generations in many of the families. In addition to these 112 families that had participated in a series of studies reported from 1937 to 1943, a special group of 291 families were included in a special genetic study. In all of the studies to be reported here, standard diagnostic criteria were used.

The background for suspicion that familial susceptibility might be a factor for consideration in the study of the natural history of rheumatic fever was similar to what has been described for tuberculosis and poliomyelitis: that is, it was based on the clinical impression of the concentration of rheumatic fever within families and in particular geographic areas that could not satisfactorily be explained by reference to known environmental circumstances or the characteristics of familiar infectious agents.

In order to explore the subject of familial susceptibility, I turn first to the observations that bear on the existence of a familial tendency and the measurement of its extent. For this purpose, in the Baltimore and New Haven studies comparisons were made of the occurrence of rheumatic fever in families of index cases and controls. The Baltimore study included data concerning: (1) siblings; (2) parents; (3) uncles and aunts; and (4) grandparents (FIGURE 2). In every comparison, individuals in rheumatic fever families were attacked in larger numbers than members of the control series; the secondary attack rate was more than 3 times as great in 3 of the 4 comparison groups. These results were corroborated by a comparison of annual attack based on person-years of life experience. Results obtained in New Haven were comparable. In this group, the high secondary attack rate among siblings of cases reflected the much longer period of observation and, accordingly, the older attained ages. Among the spouses of rheumatic fever cases and control groups, the differences were not significant. The results of both studies are consistent in showing higher attack rates among members of rheumatic fever families. Of particular interest were the comparisons among grandparents and uncles and aunts who did not share the same household or immediate environment but did share a common inheritance. The higher rates in rheumatic fever families were considered indicative of strong familial tendency. The caution was expressed however, that "... this does not eliminate the possibility that exposure (of some sort) is also an important factor" (Read *et al.*, 1938).

Further evidence relative to the existence of a familial susceptibility to rheumatic fever and its possible extent is provided by a comparison of the occurrence of rheumatic fever in sibships in which 1 or both parents are known to have the disease with sibships in which both parents are unaffected. In each of the 4 cities, the secondary attack rate among siblings (defined as the attack rate among siblings of the index case) was higher in the families where 1 or both parents had rheumatic fever than in families with 2 unaffected parents (TABLE 12). These consistent findings in 4 independent studies lend support to the hypothesis of familial susceptibility.


BALTIMORE STUDY

NEW HAVEN STUDY

* Index cases omitted

FIGURE 2. Attack ratio among relatives of rheumatic fever and control families. Data from Read, 1938, TABLES 2 and 4; Gauld, 1939, p. 213; Gray, 1952, TABLE 3.

It must be remembered that the secondary attack rates in the 4 cities are not comparable by reason of differences in age, period of observation, and other factors. The significant comparison is between the 2 types of parental classification within each study, and in this respect the studies are in agreement. In addition, in 2 of the studies there were a few families with 2 affected parents; in these families the proportion of children with rheumatic fever was higher than in the families with only 1 affected parent. The same interpretation was made in each study: that familial susceptibility was a significant factor in the occurrence of rheumatic fever.

It was plain to these investigators that the differences in familial concentration might also be accounted for by other hypotheses, such as exposure or household environment. Outbreaks of rheumatic fever have been reported in hospital wards, convalescent homes, and elsewhere, suggesting that the disease may be spread by intimate contact. It is widely believed that rheumatic fever is the result of an infection with a low degree of infectivity and that spread in families usually occurs in association with respiratory infections. Environmental factors have also been regarded as important, since the disease has been reported more frequently in association with substandard housing, especially crowding, and where food and clothing have been insufficient. In the terminology of epidemiology, there may be other agent, environment, or host factors that can account for the differences observed among offspring of affected and unaffected parents. Evidence from three lines of inquiry will be considered: (1) exposure within the family; (2) environmental differences; and (3) the factor of age.

TABLE 12

SECONDARY ATTACK RATE OF RHEUMATIC FEVER AMONG SIBLINGS IN RELATION TO DIAGNOSIS IN PARENTS

City	Secondary attack rate		Source
	Both parents not affected (%)	One or both parents affected (%)	
Belfast, Ireland	6	12	Stevenson, 1955; TABLE 1
Baltimore, Md.	13	22	Gauld, 1940; TABLE 1
New Haven, Conn.	21	31	Gray, 1952; TABLE 9
New York, N.Y.	22	44	Wilson, 1943; TABLE 5

If rheumatic fever is an infection that may be transferred from person to person, its occurrence may be related to various types and sources of exposure. For purposes of investigation, recurrences may be regarded as exacerbations of existing disease, in which case the principal end point of observation is the onset of new cases or, alternatively, the disease may have a short incubation period, in which case recurrences represent new infections. In the Baltimore study the occurrence of new cases among siblings of an index case (measured as the annual attack rate in per cent) was significantly higher following exposure to an acute episode within the family ($P = 0.02$). It was also noted that the age-specific attack rates were generally higher in families with a history of rheumatic fever in 1 or both parents, although the experience within each age group was not large enough to give statistical significance to the differences. It is of interest that the incidence is 60 to 76 per cent higher among children of affected parents as compared with children of unaffected parents, and that this ratio is not increased after association with an active case (TABLE 13). Furthermore, analysis of the occurrence of multiple cases within the same family did not show a concentration of new cases following an acute episode in a member of the family. The evidence suggested that it might be a matter of years after the disease had occurred in 1 member of the family before another was attacked.

Analysis of recurrences of rheumatic fever gave results consistent with the foregoing. On the hypothesis of communicability, it might have been predicted that more cases would occur after exposure to an active case within the family than would occur without such exposure, and that there would be more years of active disease in families with several children than in families with only 1 or 2 children. In fact, however, neither of these expectations was realized. The families studied in Baltimore and New York failed to show any tendency for new or recurrent cases to occur within short time intervals following exposure to acute episodes. In both cities it was observed that new cases and recurrences were more frequent in families with an affected parent than without one. Considered in conjunction with TABLE 13, these observations are consistent with the interpretation that "... heredity plays a definite role in the etiology of the disease" (Gauld and Read, 1940). Furthermore, if infection is a factor, the results of exposure in the household should be measured not in weeks or months but in years.

TABLE 13

ANNUAL ATTACK RATE OF RHEUMATIC FEVER AMONG SIBLINGS WITH DIFFERING PARENTAL HISTORY; BEFORE AND AFTER ASSOCIATION WITH AN ACTIVE CASE

	Annual attack rate (new cases)		
	No parental history (%)	Parental history (%)	Ratio increase (%)
Before association with an active case	0.98	1.73	176
After association with an active case	2.50	4.00	160
Ratio increase	255	231	
Probability	0.02	0.02	> 0.05

Data from Gauld and Read, 1940, p. 395–6.

Turning now to the role of the household environment, it will be recalled that in the New Haven study, families with rheumatic fever and those used as controls were selected among local residents attending New Haven Hospital for trivial as well as serious ailments. From 1930 to 1931, the income levels were low and closely similar in both groups of families, and there was little difference in family size; however, poor housing and crowding within the home were found to be significantly greater in the affected families. There was general improvement in housing and crowding during subsequent years, so that by 1948 and 1949, differences in living conditions between the 2 groups were small.

The economic levels of the families under observation in New York Hospital varied from dire poverty to middle-class comfort. Higher income families were not represented in the children attending the cardiac clinic. The influence of environmental factors may be estimated from the occurrence of new cases in these families. There was little difference in the occurrence of rheumatic fever among the siblings of subjects living under relatively favorable and of those living under unfavorable conditions: 37 per cent compared with 33 per cent (Wilson, 1940). In the group living under least favorable circumstances, the rates were somewhat higher: 44 per cent. Although this experience was not large enough for the difference to be significant, the latter group included a greater proportion of children who had not yet passed the critical age period.

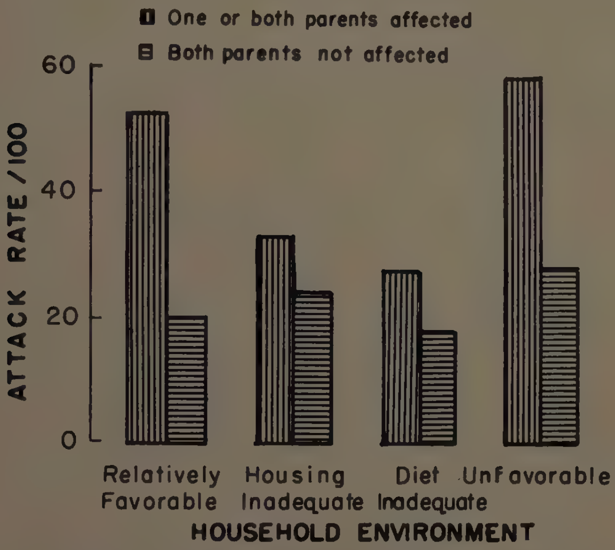


FIGURE 3. Attack rate of rheumatic fever among siblings with different parental history and household environment. Data from Wilson, 1940, TABLE 7.

Comparing total rheumatic activity, including recurrent attacks, the rate was consistently higher in families living under less favorable conditions.

Of particular interest for our discussion is a comparison of the household environment in relation to the presence or absence of rheumatic fever among the parents (FIGURE 3). In every environmental grouping, children of affected parents had a significantly higher percentage of rheumatic fever than the children of unaffected parents; the lowest percentage in the former was higher than that observed in any environmental group of the latter. This is in marked contrast to the small differences attributable to the effect of environment alone.

Although more cases occurred when rheumatic fever was present in a parent than when it was absent, definite conclusions on the role of environment do not appear possible until data are available for families living in better circumstances than those represented in these studies. It seems probable, however, that the presence of markedly unfavorable diet, housing, and other household conditions adversely affect the course of the disease.

In the study of communicable diseases, comparison of the age of onset among children within a family provides information regarding the pattern of transmission. In acute communicable diseases of childhood, secondary cases within families occur at younger ages than primary cases; whereas the age of onset in single-case families is the same as for primary cases. For measles, as shown in TABLE 14, the average age of onset of secondary cases was 3.7 years, nearly 2 years younger than for primary cases in the same families. For rheumatic fever, in contrast, there was no significant difference in average age of onset of primary and secondary cases in New York and New Haven. Tabulations of cases in families in relation to birth order followed a similar pattern. For poliomyelitis the median age of onset in second-born children was more than 2 years earlier than in first-born; among later-born children the onset was 4 years earlier than for first-born. However, in families with multiple cases of rheumatic fever, the age of onset was similar for each birth order. Thus there seems to be a characteristic age of onset for rheumatic fever. These observations do not support the hypothesis that rheumatic fever is passed back and forth from older to younger children in a family according to the characteristic pattern of infectious diseases.

TABLE 14

AGE OF ONSET IN FAMILIES: FOR MEASLES, POLIOMYELITIS, AND RHEUMATIC FEVER

Primary and secondary cases in families	Average age of onset (years)		
	Measles	Rheumatic fever	
		New York	New Haven
Single-case families	5.3	5.7	7.9
Primary cases	5.6	5.9	8.7
Secondary cases	3.7	6.9	10.7
First-born and later-born cases	**Median age of onset (years)**		
	Poliomyelitis	**Rheumatic fever (New York)**	
First-born	7.0	5.5	
Second-born	4.5	5.1	
Others	3.0	5.6	

Data from Wilson, 1940, p. 36–38; Gray *et al.*, 1952, p. 406 by interpolation.

Age-specific secondary attack rates were lower in families with unaffected parents than in families where 1 or both parents were affected (TABLE 15). In the first group, the attack rate rose to a maximum of less than 8 per 100 years of life experience, whereas in the latter the peak was about twice as high. Beginning in the ninth year, the secondary attack rate fell off rapidly in both groups. Similar results were reported in the Baltimore study. These observations are consistent with the hypothesis of familial susceptibility. The possibility that the higher rate in the second group might be due to exposure from a parent with rheumatic fever appears unlikely because the standardized attack rates prior to the occurrence of the first case among siblings was only slightly higher in families with an affected parent.

There is additional evidence that age-susceptibility may be a characteristic feature in the onset of rheumatic fever. Children having their first association with active cases after their eighth birthday were attacked infrequently in comparison with children who had their first association with an active case at an earlier age. Similarly, exposure during the first year of life to an active case did not result in a higher attack rate than occurred among children exposed at later ages. The interpretation of these findings is uncertain; it may reflect some unknown host characteristic other than general familial susceptibility.

In summarizing the data regarding communicability, household environment, and age of onset, it seems to me that they lend additional support to the hypothesis that there is a familial susceptibility to rheumatic fever. As has been seen, there is little evidence suggesting that exposure may be a factor, and it appears unlikely that the disease spreads in families during short intervals following exposure to an acute episode. Poor home environment tends to increase the severity of rheumatic fever and may also be a factor in the onset of the disease. Furthermore, the age characteristics appear consistent with the interpretation of familial susceptibility.

TABLE 15
AGE-SPECIFIC SECONDARY ATTACK RATES FOR RHEUMATIC FEVER AMONG SIBLINGS IN RELATION TO DIAGNOSIS IN PARENTS

Age group (years)	Both parents not affected (%)	One or both parents affected (%)
1–2	0.7	2.0
3–4	7.8	8.6
5–6	7.1	13.7
7–8	3.2	19.5
9–10	3.8	8.0
11–12	1.9	4.2
13–17	0.3	1.9

Data from Wilson, 1940, p. 43.

The usefulness of the hypothesis of familial susceptibility would be increased if the mode of inheritance could be determined. For this purpose, the data from the New Haven and New York studies are appropriate. In both studies, families were under observation long enough for the great majority of the children to have passed the age-susceptible period. Inasmuch as the individual pedigrees revealed little or no evidence suggestive of dominant inheritance or sex linkage, the hypothesis of recessive inheritance was investigated.

These families, it has been noted, had been selected through affected children. The proband method of analysis was used in both studies although the method of ascertainment could not be accurately determined at the time of genetic analysis. In both studies the results were in fair agreement with the predicted values; there was an excess of observed cases (TABLE 16). For the New York study, the difference between observed and predicted cases was on the borderline of significance in 1 mating combination.

A further test of this hypothesis was therefore undertaken. For this purpose, a new series of 291 families was gathered, and selection was made through a parent in order to simplify the analysis. The diagnosis of the mate, although known in some cases, was not considered in the selection of families. The unselected mates were examined, as well as their parents and siblings, wherever possible. The period of observation of the families averaged 10 years for the entire series. From this information, estimates of the expected occurrence among the siblings were made on the hypothesis of recessive inheritance.

TABLE 16

RECESSIVE INHERITANCE IN RHEUMATIC FEVER: COMPARISON OF OBSERVED AND EXPECTED CASES IN FAMILIES ASCERTAINED THROUGH AN AFFECTED SIBLING

	New Haven cases		New York cases	
	Observed	Expected*	Observed	Expected*
Both parents unaffected	59	55	101	90 ± 5.3
One parent affected	16	15	117	112 ± 6.4
Both parents affected	6	9	14	15

* Proband method.
Data from Gray *et al.*, 1952, p. 409; Wilson *et al.*, 1943, TABLE 5.

TABLE 17

RECESSIVE INHERITANCE IN RHEUMATIC FEVER: COMPARISON OF OBSERVED AND EXPECTED CASES IN FAMILIES ASCERTAINED THROUGH AN AFFECTED PARENT

Mating	Families	Siblings		Affected sibling	
		Total	Under 6 years	Observed	Expected*
No affected offspring expected	239	525	105	3	0
+ × −	183	399	80	2	0
Cp × −	30	65	13	0	0
− × −	26	61	12	1	0
Some affected offspring expected	52	121	14	40	49
+ × +	8	15	2	14	15
+ × Cp	7	20	0	10	10
+ × Co	28	63	8	11	21
Cp × Cp	2	4	0	1	1
Co × Co	7	19	4	4	2

* Based on examination and family history of parents.
Note: + = affected; − = unaffected, with no cases in close relatives; Cp = unaffected, with an affected parent; and Co = unaffected, with an affected close relative, other than a parent.
Data from Wilson and Schweitzer, 1954, TABLE 3.

Predictions for each family were made without knowledge of the clinical observations that were gathered for the children, and no comparisons were made between predictions and observations until all observations and estimates had been recorded.

The principal results are shown in TABLE 17. In 239 families, where 1 parent was not affected and rheumatic fever had not been reported among his parents or close kin, no cases were expected, and only 3 cases were found among 525 siblings. In the 52 families where 49 children were expected to come down with rheumatic fever during childhood, 40 cases were observed. In view of our present inability to identify "susceptible" individuals, the occurrence of 3 cases in the group where none was expected is not remarkable. Since 80 per cent of the children in these families have passed the age of maximum incidence of rheumatic fever, only a few additional cases may be anticipated. Accordingly, the results obtained in this series are considered to be consistent with the hypothesis of recessive inheritance.

Discussion

"What are the practical goals of genetic studies in communicable disease?" This question was asked only a short time ago when I was drawing together ideas for this paper, and I shall attempt an answer. Prevention of disease through the interception of causes is the ultimate goal of epidemiology. The more accurately we can specify the characteristics and functions of the multiple causes of disease, the better we can improve measures for prevention.

In this complex, the search for genetic determinants has a specific application. Knowledge of the chemistry of heredity and the biological behavior of genetic determinants is rapidly increasing, and this knowledge will find application as it becomes possible to be more explicit in describing the genetic causes for more diseases. When it is known that there is a general pattern indicative of familial susceptibility, we may be assisted in identifying groups that are at increased risk. With this more efficient means of detecting disease in earlier stages, treatment measures become more effective. When more is known about the mode of interaction of genetic determinants with agent and environmental factors in a given disease, it will be possible to develop measures for modifying these interactions in ways that will ultimately prevent the occurrence of that disease. This is the rationale that guides investigators in the study of all the causes of disease and applies with equal force to genetic causes.

This is of particular interest in the three diseases under discussion for, as far as can be judged today, it will never again be possible to replicate most of the studies I have described, particularly those based on long-term observations. The prevalence of poliomyelitis, tuberculosis, and rheumatic fever is declining rapidly in many parts of the world. This is due in part to the introduction of measures for specific protection against each disease and in equal or larger part to improvement in sanitation, diet, and the general conditions of human society. It is for this reason that a detailed acquaintance with the epidemiology of any disease is a prerequisite to genetic studies of that disease, as Gelfand (1958) has so trenchantly indicated. With this introduction, I proceed to a critical appraisal of the data for each of the diseases and their more general implications.

I have been asked to consider the question: "How heavily can we weigh the role of genetic determinants in each of the diseases reviewed?"

For discussion purposes I might identify several levels of the information available at any time for the purpose of evaluating the role of genetic determinants in communicable disease. I should describe the first level as *suspicion of familial susceptibility*. The evidence for this is based on studies of familial aggregation in relation to other factors known or believed to contribute to the occurrence of the particular disease; the evidence must be of sufficient variety and detail to justify the inference that familial susceptibility is also involved. The second level I should call *interaction*. For this, studies should be made in which other important factors as well as familial susceptibility are considered together. Evidence of differences in the effects of other variables in relation to possible genetic differences, when sufficiently detailed so as to clarify the interrelationships, would support the inference of interaction. The third level, *indirect identification*, is based on the genetic analysis of the distribution of affected and unaffected individuals in families. Evidence would consist in agreement between observed and expected occurrence in families, where the expected values are derived from a postulated mechanism of inheritance. This mechanism would also have to be consistent with direct observation in the individual families, and there should be no unexplained exceptions or wide deviations. The fourth level is *direct identification* and requires the recognition of the postulated susceptibility by means of some specific characteristics, which may be superficial or internal anatomic traits, or identifiable by cytologic, chemical, or physiologic tests. Since this stage has not yet been reached for any communicable disease, further elaboration is not needed for the present discussion.

To apply this yardstick to any disease, data of several types must be reviewed. For example, where an agent has been identified, the characteristics and mode of interaction of the agent and man must be considered. If this is not done, it will not be possible to decide whether observations suggesting familial susceptibility may not rather be due to the mode of action of the agent. Similarly, environmental circumstances known to contribute to the occurrence of a communicable disease must also be taken into account. Furthermore, additional information from more detailed studies of interaction of several factors that may enlarge the evidence pertaining to familial susceptibility should not reveal major inconsistencies with previous observations.

From this discussion it is apparent that the first three levels (suspicion of familial susceptibility, interaction, and indirect identification) are interrelated. For any disease, evidence of the mechanism of inheritance may appear prior to an understanding of the mode of interaction. When this occurs, indirect identification cannot be considered satisfactory until studies demonstrate the mode of interaction, which does not suggest that the interpretation of susceptibility is unnecessary to account for the distribution of cases. Thus evidence for the first three stages of information, although not always gathered in sequence, must be considered in the order suggested. The fourth level (direct identification) although usually last to be achieved, is less dependent on the other levels. When direct identification has been achieved, more detailed studies and practical applications can be pursued with confidence.

Turning first to poliomyelitis with these information levels in mind we must ask: How solid is the evidence for genetic determinants? Has it withstood challenge? What suggestions can be made to clarify further the role of genetic determinants in poliomyelitis?

The observations of Addair and Snyder are a solid addition to our information, and I am prepared to accept their conclusion that a pattern of inheritance was demonstrated in the particular region of West Virginia for the kindred they studied. However, the universal application of their findings is still in question. Indeed, there is need for scepticism in this respect because of the many illustrations of phenocopies of genetic diseases. Surely some cases of blindness and deafness are hereditary; the question at issue is: "How many?"

The principal criticism of the family study in Indiana is the inadequacy of the controls. Frost (1933) has pointed out that in general the attack rate in families does not distinguish between the risk resulting from familial susceptibility and the risks due to differences in environment or exposure. Gelfand has given reasons why neither the opportunity for infection nor the probability of developing paralytic sequelae are uniformly distributed in a large area such as northern Indiana and he has emphasized the need for carefully matched controls.

The twin studies of Herndon and Jennings on poliomyelitis are among the best that have been conducted on any disease, and they were published with sufficient detail to make their contribution of lasting value. Lilienfeld (1954) has suggested that the differences between the two types of twins might be due to the presence of a larger number of older siblings in the families of dizygotic twins, because the incidence of dizygotic twins increases with birth order, whereas the incidence of monozygotic twins does not. If this were the case, the dizygotic twins might have been infected at an earlier age than the monozygotic twins, with less chance of developing paralytic poliomyelitis. Fortunately, the published data permitted an examination of this point. Lilienfeld's criticism is not sustained, for the differences in concordance between dizygotic and monozygotic twins are similar whether there are very few or many older siblings in the household.

However, there are theoretical and practical problems in the evaluation of twin studies that make it difficult to decide whether differences between monozygotic and dizygotic twins can be interpreted as reflecting differences in one or a small number of genetic determinants. Since this is still the subject of debate, it merits comment. Monozygotic twins are identical not only for the one or few genes that may be hypothesized as responsible for susceptibility to a particular disease such as poliomyelitis or tuberculosis but are identical for their entire genetic constitution. In this respect, monozygotic twins can be compared to litter-mates of a long-inbred strain of mice, which serve so well in replicated experiments where minimal host differences are desired. Dizygotic twins, in contrast, are siblings with the accident of nearly simultaneous conception and delivery; these twins are genetically much less similar but to an extent that unfortunately cannot be determined. For both types of twins as ordinarily studied we do not know the extent to which groups of twins are homozygous for the specific determinants that may be responsible for susceptibility or insusceptibility to a particular disease. This is the reason that interpretation of twin differences is often clouded. There are also problems with respect to environmental similarities and differences. In addition, the classification into monozygotic and dizygotic twins is not always clear-cut. Further comments on these matters have recently been made by Osborne and DeGeorge. In brief, I find myself unable to judge to what extent differences in twin concordance with respect to poliomyelitis might be ascribed to differences in genetic determinants.

Neel (1955) has called attention to the confusion that arises when efforts are made to describe a mechanism of inheritance before the role of genetic determinants has been identified. When this is attempted, the concept of penetrance often serves to confuse rather than to clarify.

The studies of Aycock provide the best available documentation that familial susceptibility is a factor in poliomyelitis; other studies are consistent with this interpretation.

Thus I should conclude that for poliomyelitis the level of *suspicion of familial susceptibility* has been attained, but there is as yet little evidence of the interaction of this factor with other known causes of poliomyelitis. Information concerning these interactions could be obtained from family epidemiologic studies of outbreaks as was done in Indiana if controls along the lines suggested by Gelfand were included in the study design. To carry this suggestion further for future research, I might predicate the following: if sufficient evidence of interaction were obtained, studies might then be undertaken to determine the mechanism of inheritance by genetic analysis of the distribution of cases in families. An attempt might be made also to compare the nature and evolution of immunity to attenuated virus in susceptible and naturally resistant individuals. Studies of the intestinal mucosa of susceptible and resistant individuals might explain the nature of barriers to the passage of poliomyelitis virus into the circulation. Such a series of studies might then lead to identification of the reactions in which the genetic determinants for poliomyelitis may be manifest. These suggestions, however, should not be taken for more than the illustration I intended them to be; thus let me end this discussion with the comment that, at present, there is suspicion of familial susceptibility in poliomyelitis: the rest is speculation.

In tuberculosis, interpretation of the twin study of Kallman and Reisner is subject to the same difficulties that I have indicated for the poliomyelitis twin studies. In addition, the absence of controls for environmental differences and lack of information on the amount of exposure within the families must lead to hesitancy in interpreting these studies. The analysis by Planansky and Allen of the outcome of tuberculosis in concordant twins is free of this criticism, since the circumstances of treatment were known for all subjects, and the majority were observed until the clinical course of disease was completed.

In the Williamson County family investigation, controls were available for comparison although not always under the same close observation. In this study, information on exposure was available with respect to the sputum-status of tuberculosis patients. Exposure to risk was measured by application of life-table techniques to the analysis of the data. A weakness in these studies, however, was the lack of information on environmental factors, particularly nutrition and housing. There is a fair likelihood that the effects interpreted as interaction between familial susceptibility and the tubercle bacillus may instead reflect interaction between the bacillus and environmental factors.

The terms susceptibility and resistance, as used in many studies, are not clearly defined. Confusion occurs because of failure to discriminate between susceptibility that may be genetically determined and susceptibility that may result from intra-uterine or postnatal experience. Difficulty also arises because susceptibility and resistance are commonly regarded as the extremes of one scale. If the interaction of only two factors were being measured, this concept might suffice. This is not usually the case either in laboratory animals, as Schneider (1956*a*) has so persuasively argued, or in human diseases such as tuberculosis. Perhaps the difficulty is that this model is inadequate to describe the interrelations of multiple factors. An effect that might be described as relative resistance, with regard to agent-host interaction, might be described quite differently if an environmental factor were added. This argument has direct relevance to the various published observations on tuberculosis. Puffer cited the argument that since very few persons escape infection, clinical or subclinical, the escape from disease must be credited to genetic resistance rather than to avoidance of exposure. I do not agree with this view, because the postulated resistance might be equally the result of improvement in either nutrition or living conditions, or of other factors. Evidence is lacking as to whether the imputed difference in susceptibility is genetic or nongenetic; it seems in fact that both may be present in a given population at the same time. This is implicit in the observation of Frost (1939) that the mortality from tuberculosis in the United States has been declining since 1870 or before, which is prior to the introduction of measures aimed at prevention and control.

Planansky and Allen observed that the onset and early stages of tuberculosis in concordant monozygotic twins were quite similar but that the clinical course and final outcome were often different and varied with hospitalization and other factors. Their interpretation was that the factors responsible for resistance to the course of tuberculosis were quite apart from those governing resistance to the initiation of infection. This interpretation for tuberculosis is consistent with what has been postulated in other human diseases and also in studies of dietary and genetic factors in infectious diseases of laboratory animals. This illustrates rather pointedly that the concept of a generalized susceptibility factor for disease tends to confuse rather than to clarify.

In summary, the evidence seems persuasive that familial susceptibility is one of the factors involved in tuberculosis, whereas evidence for the interaction with other factors is inadequate. Corroborative studies are needed in which environmental factors are also considered. Further studies to evaluate the activity of possible genetic determinants in tuberculosis might take into consideration sex differences in occurrence and mortality, the relation between exposure and onset under conditions of observation in long-term studies, and the nature of the resistance in highly immune individuals.

For rheumatic fever, detailed and extensive observations were made during the course of the four independent investigations.

Evidence for *suspicion of familial susceptibility* is based on the comparison of relatives in affected and control families and, in addition, comparison of the secondary attack rate among siblings with different parental diagnosis. Corroboration of each finding was reported.

One feature of these observations has been questioned by Stevenson and Cheeseman (1956), who noted that secondary cases occurred less frequently in Belfast than in any of the cities in the United States and that the proportion of affected parents was also lower. The Belfast families were derived from consecutive admissions to a children's cardiac clinic; comparison is possible with the Baltimore study, in which a similar procedure was followed. In 1938, Read *et al.* had noted that the percentages of affected siblings and parents observed in Baltimore were higher than had been reported from hospitals in London and Glasgow. They suggested that ". . . there may be real differences associated with the conditions peculiar to this locality or it may be a difference dependent upon the methods of sampling or the extent of the different investigations." The rate among siblings in Belfast fell between the rates reported in London and Glasgow. Whatever may be the explanation of these differences, the significant comparison is between the children of affected and unaffected parents. The results of this comparison were the same in Belfast as in the other cities, that is, the secondary attack rates were higher among the children of affected parents.

Evidence of *interaction* of suspected genetic determinants with agent and environmental factors was obtained in observations of exposure to active manifestations, to differences in household environment of several types, and in relation to age.

Modern theories of the role of infection in triggering attacks of rheumatic fever have been derived chiefly from studies of recurrent attacks, and it is not known whether recurrences represent new infections or exacerbations of existing disease. Until additional information is available on this and other aspects of host-agent interaction, the interpretation of attack rates in rheumatic fever will remain uncertain.

The relation of age of onset to exposure was considered by Gauld and Read (1940). They suggested that the age of onset could be regarded as an indicator of the interaction of genetic and environmental factors in rheumatic fever. If acute manifestations among members of a family occurred at the same age, this could be taken as evidence that the onset was due solely to heredity. If, however, in addition to heredity there were environmental factors of any type (for example, infection or nutrition), a certain amount of clustering of additional cases would be expected after one member of the family had developed acute manifestations. Data have been presented that are consistent with both of these interpretations. Although all the data are consistent with the interpretation that genetic determinants are therefore significant factors in the etiology of rheumatic fever, the apparent inconsistency with respect to the role of exposure makes it important to seek an explanation.

The evidence for *indirect identification* of genetic determinants in rheumatic fever is based on data from 2 clinics, in which ascertainment was made through an affected sibling, and on data of 291 families in which ascertainment was through an affected parent. Several criticisms have been directed at the data ascertained through an affected sibling. One criticism refers to a shortage of cases among the offspring of 2 affected parents, where all children were expected to have rheumatic fever; in 3 families with 9 children, only 6 cases were observed. The deficiency of 3 cases, while of interest, would not appear to be a serious deviation.

The second criticism, already mentioned, referring to the excess of cases observed, above the number expected on the hypothesis of recessive inheritance, is more important. Since I had the responsibility for the genetic analysis of the data collected at New York Hospital, I feel free to admit that there was great temptation to attribute deviations to errors in diagnosis, particularly in several adults whose childhood history was vague or inconclusive, or to ascribe the deviations to use of the proband method, especially since the method of ascertainment could not be described. However, both of these "explanations" appeared distasteful to me, and I was glad of the opportunity to participate in a further study in which ascertainment was through an affected parent. In so far as I know the data from the last genetic study have not as yet been challenged, although I am still optimistic enough to hope that someone will do so. However, for the present, my opinion is that the data on rheumatic fever as a whole are consistent with the hypothesis of recessive inheritance. Since this conclusion is based on indirect evidence, the possibility of a more complex pattern involving several genes cannot be excluded. The need for this type of secondary interpretation would be important if, in a given mating combination, a considerable number of cases occurred where none were expected on the hypothesis of recessive inheritance. Fortunately, we have not been confronted with this situation. Perhaps you will recall from TABLE 17 that there were only 3 exceptions among 525 children in families where none were expected. For this reason it does not seem necessary to qualify the interpretation of recessive inheritance.

In summary, indirect identification of recessive inheritance of susceptibility to rheumatic fever appears to be documented within the limits of this type of analysis. Only when susceptible individuals can be identified by means of specific characteristics or tests will it be possible to make a direct examination of the role of heredity. Studies are needed to describe possible anatomic characteristics or physiologic tests that could serve to identify individuals, who at present can be only indirectly implicated. Efforts in this direction have begun. Starting from certain theoretical considerations, the suggestion has been made that the secretor status of rheumatic fever patients might be explored (Glynn *et al.*, 1956). Studies have also been started to compare certain hormonal, chemical, and immunological characteristics of susceptible and insusceptible children during early childhood (Wilson, 1948). Further studies of the relation of infection to the onset of rheumatic fever would help dispel the inconsistencies in attack rates to which I have referred and might lead to an understanding of the factors and circumstances that trigger the onset of this disease.

My own experience in studies of genetic factors in disease has led me to emphasize the need for studies that depend on familial aggregation for suspicion of genetic determinants. This limits the field of study to conditions affected by single major genes or, at most, by a small number of additive genes. Other models based on polygenes are under active study and controversy (Penrose, 1953; Snyder, 1954). Discussions of this topic occur elsewhere in these pages.

In closing, I must mention that one purpose of my discussion has not been stated; I nevertheless trust it has been made plain in this paper. Opportunities for productive collaboration of geneticists in epidemiologic research are abundant. If progress has been slow, as witnessed by the paucity of genetic research in communicable disease, then this is the price of isolation and fragmentation of research efforts. Perhaps greater attention to the study of familial epidemiology will enlarge opportunities for assessing the role of genetic determinants in disease. Although I have emphasized communicable diseases, many of the techniques mentioned can be adapted to the study of other diseases. If epidemiology is the study of the distribution and causes of disease in human society, for the purpose of prevention and control, then surely there must be a need in epidemiology for geneticists.

Summary

The purpose of this paper has been to review the contributions of recent genetic studies of communicable diseases and to assess the progress that has been made toward identifying the part played by genetic determinants in the natural history of communicable disease.

The types of studies that have been useful in exploring the role of genetic determinants include surveys of familial aggregation, twin concordance, familial epidemiology, genetic analysis, and planned long-term studies.

For poliomyelitis, the hypothesis of familial susceptibility rests on the familial concentration and twin concordance. Additional studies are needed of the association of familial susceptibility with other factors that are also responsible for the occurrence of this disease.

In tuberculosis, studies have included environmental factors as well as familial susceptibility, which have yielded certain information regarding their interaction. More information is needed regarding the extent of genetic influence and circumstances of interaction as well as identification of the distribution and behavior of genetic determinants.

Studies of the interaction of genetic determinants with environmental and other host characteristics in rheumatic fever have yielded more information on the role of determinants than is available for other communicable diseases. Recently, studies of the interaction of host and agent have been initiated, and investigation of the biochemistry of the determinants can now be considered.

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GENETIC RESISTANCE AND SUSCEPTIBILITY TO INFECTIOUS DISEASE: A SYNTHESIS

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The purpose of the papers in Part III of this monograph has been to find, describe, and evaluate genetic parameters in infectious disease.

I have always believed that investigation should begin in the laboratory, where the experimenter has at his command mating systems, environments, the adjustment of dosages of pathogenic agents, and the arrangement of host and pathogen genotypes: that is, the whole panoply of the things that have come to be recognized as important factors that must be brought to bear on the examination and delineation of the genetic components operating in the problem of infectious disease.

Oddly enough, although the subject seems a little more complicated in the light of these papers (since we are considering population interactions), in some ways the situation is clearer. Certainly the opportunities for manipulation here are greater. The analysis has been more complicated but, at the same time, I believe it is possible to arrive at a position strong enough on which to take a one-legged stand.

In the laboratory, as Gowen mentions elsewhere in these pages, it has been demonstrated that there are genetic determinations of infectious disease of a kind, magnificent enough to embrace the whole spectrum of experience.

I should be remiss if I did not remark in passing that all of this came about for a variety of reasons. One is the search for pure knowledge. Another important and motivating influence, however, was the circumstances of the great influenza pandemic of 1918 and 1919 when information following the golden age of bacteriology was inadequate for coping with a world-wide pandemic. That challenge had several responses. One of them was the establishment of a school of experimental epidemiology that, in the hands of Webster led, in the United States, to an appreciation of host factors. The first of these to be brought forward on any convincing basis was the genetic parameter. I think one should not forget that there was more to this enterprise than simply injecting stocks of mice and observing the results. There was a penetration into the world of herds, or populations. When Webster had synthesized his stocks of inbred selected susceptibles and resistants, he was able to synthesize populations of known frequencies of known genotypes. Under such conditions, when the pathogen was let loose, the results followed as predicted.

I think it wise to remember William James's dictum that science has two goals: to conceive simply and to predict; when these are achieved then there is science.

Gowen's analysis, with his unique description of how the same genotype of animal can be resistant to one disease and susceptible to another, was also duplicated in the infectious diseases used in Webster's experiments wherein St. Louis encephalitis virus and *Salmonella enteritidis* were both used as test agents. Webster synthesized by inbreeding and selection the four combination stocks that are possible in terms of resistance and susceptibility to these two diseases. For once and for all, this disposed of ideas of a panresistance phenomenon. Geneticists deal with specificities, but with a proviso. As Gowen has pointed out, the important fact is that present knowledge stands somewhere between the exquisite specificities of the immunologists, where every pathogen strain has its own unique immunological consequences, and the generalized Hippocratic panresistance idea. The genetic factors discussed elsewhere in this monograph seem to deal with hosts and pathogens as taxonomic groups. I think it may be anticipated that, for example, as more is learned in dealing with the salmonelloses in these terms it will be possible to include *all* of the salmonelloses in chickens, in mice, and in man. Specificities, in a word, will fall into groups. If the world is not as simple as we should like, neither is it as complicated as we might fear.

There is another parameter that I cannot resist discussing in this connection: that is the effect of host nutrition. I allude to it only because the papers in Part III deal with populations. They are considered now in terms of genetic parameters, but it must be remembered that the operation of the geneticist is "directed breeding" and, as society is now constructed, such a program is not easily put into effect. Indeed I think its initiation is in the very, very distant future. Man in his genetic aspects, however, is still capable of being analyzed in terms of something yet to be mentioned: the Hardy-Weinberg equilibrium in which matings occur at random. That is the situation with which we ultimately must deal. Man, to look at him from another facet, is an eternal meddler. He has not yet meddled with his genes. That he will do so, I am sure; but at present, in this cross section of evolutionary time and in past evolutionary history, he has been an environmental meddler. Man almost lives out his days now in a man-made environment, but the phenotypic consequences of those environments still flow from interactions with the genotype. It is for this reason that I say genetics is central in biology. Schweitzer elsewhere in this monograph questions this; I reaffirm it.

Schweitzer is correct, however, when he says we must remember multicauses. It is also true that when we have multicauses we must arrange them in hierarchies. Such causes are not all equal. The day may well come when there will be monographs on how to agree on arranging those hierarchies. There must be hierarchies and, moreover, their ordering demands value judgements. Value judgements can be debated but not proved. For that reason I can debate with Schweitzer, but I can never prove him wrong, or right.

Allison gives us a beautiful example of something I think should be remembered for a long time. Taking off from the laboratory and moving into the field, he uses the powers of biochemical resolution to their present historical limit. He moves from a single amino acid in the hemoglobin molecule to a map of the world where, in malarial regions, the balanced polymorphism in the sickle-cell anemia story played its role. It is an impressive story.

I think Allison would not object if I said that the problem was not so much that the heterozygotes were more resistant but that they were less susceptible. This I suggest on the basis that the altered hemoglobins are somewhat less satisfactory for the *Plasmodium* in its own nutritional problems as it lives out its short span in the erythrocyte that it has parasitized. Rudzinska and Trager have recently shown by electron microscopy, for example, that the *Plasmodium* does not digest by excretory enzymes the hemoglobin in which it finds itself swimming. It drinks it by a process of pinocytosis. It drinks the hemoglobin: whether it gets a stomach-ache or not, I suppose, now becomes a matter of some consequence.

The natural operation is described elsewhere in these pages by Allison, who discusses what reduced susceptibility can mean in the increase in frequency in certain phenotypes in an endemic disease area. Here, too, there is a price to be paid. The hidden price, worth paying in the face of malaria, becomes automatically evident when the malaria is withdrawn and the heterozygote sickler has lost its advantage. I can only repeat: the subtleties of this story are a biological tour de force.

Schweitzer makes the best of things as they are in several epidemiological problems involving infectious diseases of great importance. Schweitzer has, I think, set up some valuable criteria in terms of the path that must be taken toward certitude. He has suggested what we should look for or expect: (1) suspicions of familial susceptibility; (2) interaction (nutrition would be one of the factors that would enter here); (3) indirect identification (wherein some predictions became verifiable); and (4) direct identification when the whole is explained in terms of the biochemical details of the phenotype.

Poliomyelitis and tuberculosis criteria are far from certitude. According to Schweitzer's classification they remain at the first level: suspicions of familial susceptibility.

Rheumatic fever, however, Schweitzer carries through to the third level: indirect identification. Schweitzer's paper is valuable in showing how epidemiological data can lead to a clear view of the operation of genetic determinants in infectious disease in the human herd.

I think that in general Part III of this monograph presents to students of infectious diseases: (1) evidence for genetic parameters in such diseases; (2) the ways in which such parameters may be sought; and (3) the nature of the dialogue that precedes understanding.

DISCUSSION: PART III

J. H. **Whitlock** (*Veterinary College, Cornell University, Ithaca, N. Y.*): My colleagues and I have used a system somewhat different from that used by most workers reporting in these pages. Our system possesses some interesting facets, not because the situation involved is complex but, from one standpoint, because it is simple.

Our system, roughly, is this: we have produced in sheep for 12 years a continually recycling natural epidemic of an acute helminthic disease. The susceptible lambs are born within a month of each other. They are pastured with the carrier dams at the same time each spring. They contract the disease at the same time each summer. For some time we have been studying the various factors in the disease.

Actually this is a displaced tropical disease. The chief pathogen, *Haemonchus contortus*, is a bloodsucking helminth that is very climate-sensitive in the free-living phase, so that the disease terminates automatically each fall.

Recently we have begun to identify satisfactorily some of the phenotypes that occur. One of these phenotypes is simple, at least on the surface, and is of interest to those who deal with climatic problems.

We take many hematocrits in series on all breeds of animals because this is the quickest, easiest way to determine whether they are anemic, and thereafter to follow the course of the anemia. We found that there is a double oscillation during the period of a year in these hematocrit values, oscillations that are of considerable magnitude. One maximum is reached in winter; one minimum is reached in late August (there is another maximum and another minimum between the two mentioned, but that is not relevant here). Our finding compares favorably with findings reported in some of the Japanese work on seasonal influence on the so-called standard blood values in humans.[1]

The point I desire to emphasize is that, as we have recently shown, the maximum hematocrit value is an inherited characteristic, for we have demonstrated a highly significant regression within sires of the maximum-lamb hematocrits on the maximum-dam hematocrits.

Superficially this is a simple thing, but its importance is this: if the Japanese workers are correct, the adaptation to summer involves an increase in the plasma and a decrease in the hematocrit values; an adaptation to cold involves the reverse.

If this is true, and if the challenge that the parasite presents to the host is exactly quantitative (this is true within certain limitations), an individual who is genetically predetermined to have a low-peak hematocrit value is in an unsatisfactory status to meet the bloodsucking parasitic challenge; whereas an individual who possesses a relatively high hematocrit value is in a relatively adequate state.

On the other hand (if the Japanese workers are right), this adaptation to a hot climate that consists of a low hematocrit value should present a biological advantage to an individual genetically determined to possess relatively low values so that, in this search for an individual that can survive in the type of climate Lee mentions, there is a real dilemma. Lee is trying to produce an individual adapted to a warm climate with these low, genetically determined, hematocrit values and, if he does produce this individual, he will produce one that because of simple quantitative relationships is highly susceptible to disease caused by bloodsucking pathogens.

KURT HIRSCHHORN: I suggest a different system of approach. We have used in our laboratory a system of tissue culture in attempting to find, within cell lines, individuals that vary in infectious resistance and susceptibility. We used an aneuploid cell line derived from a normal human synovial biopsy, within which we selected for complete resistance to polio type 1 virus infection within approximately 15 to 16 generations of selection.[2] There is a slow return toward susceptibility after approximately 66 additional generations. However, if one exposes this line to ultraviolet irradiation at the time that resistance is first achieved, susceptibility returns after approximately 12 generations to a considerably greater degree than the spontaneous return.

My next comment relates to a disease thought to be of an infectious nature: acute leukemia. This suggests another possible mechanism involved in susceptibility and resistance. We know that acute leukemia occurs frequently in two types of individuals: irradiated individuals and mongols.

I shall bypass the irradiation problem, although recently it has been shown that even small doses of irradiation can cause severe damage to leukocyte chromosome morphology.

However, we[3] have recently studied a 10-year-old mongol child (by all criteria a classical mongol) who became ill with acute leukemia.

Upon studying the chromosomes in the peripheral blood of this child by leukocyte culture, we discovered that chromosomally he was not a typical mongol because he had 46 chromosomes. Two of the small acrocentric chromosomes that were expected apparently had become translocated on each other, and an extra small metacentric chromosome was found.

Possibly the loss of genetic material associated with such translocation, or some similar mechanism, may cause certain mongols to become more susceptible to diseases such as leukemia.

HSIA: I am interested in Allison's reference to the possible interaction, or at least the effect, of the sickle cell, the thalassemia, and the glucose-6-phosphate dehydrogenase genes each of which appeared to have some definite relationship to the malarial parasite.

Why do these particular defects appear in different areas regionally, and what, if anything, do they have to do with each other? Of course the relationship between the sickle cell, thalassemia, and the clinical syndrome is well known.

Jean Naylor in our laboratory investigated the clinical pattern of the individual who is both homozygous for the sickle-cell gene and also homozygous for the glucose-6-phosphate dehydrogenase gene.

She surveyed 100 individuals known to be homozygous for the production of sickle-cell hemoglobin (SS). These individuals were then measured for glucose-6-phosphate dehydrogenase deficiency. The percentage of both males and females that one would expect to be homozygous for these deficiences was the same as in the Negro population as a whole. Furthermore, the clinical syndrome in these individuals was entirely that of SS, and the glucose-6 did not appear to exert any genetic effect, at least in the quiescent stage. Red cell survival studies on these individuals showed their survival time to be identical with that of an SS individual under the same circumstances. This verifies previous observations that red cell survival time of the glucose-6-phosphate-dehydrogenase-deficient people who are not at the time exposed to primaquine or a similar agent is the same as that of a normal individual.

LEVENE: Schweitzer mentions that in some respects monozygotic twins are similar to litter mates in inbred populations of laboratory animals. I am not sure that this analogy is a happy one.

If the animals are inbred, homozygosity is increased. However, there are a number of genes that are still segregating, and therefore litter mates are apt to differ with respect to a number of genetic traits, whereas, of course, monozygotic twins are identical.

In spite of the fact that the inbreeding is not completely successful, the highly inbred line still is inbred; monozygotic twins, however, are fully outbred, and inbreeding generally results in some loss of vigor and some loss of homeostasis, even though the inbred line is a good one.

MORTON D. SCHWEITZER (*School of Public Health and Administrative Medicine, Columbia University, New York, N. Y.*): I refer to Allison's comment about the persistence of the sickling gene. I wonder how many generations have been studied.

Allison also referred to the known influence of diet on malaria. I wonder to what extent the interaction of the genetic and dietary factors affects studies in these populations.

LEVENE: I shall speak for Allison. I do not know the answer to the second part of the question, about the diet. As to how long these genes have been maintained in their present frequency: sickling has been known for only a short time. Only in this generation has sickling been studied. We have indirect evidence with respect to it. For example, sickling exists in Negroes who have been in the United States for some time. It also continues to exist in northern Negroes where the climate is not malarious. These Negroes have a lower rate of sickling than those in West Africa, where most of the slaves originated.

On the other hand, these Negroes also have considerable white mixture, and the decline in sickling approximates the rate one would expect (1) from natural selection in an environment where there is no malaria, and (2) from some dilution with white Caucasian genes.

In parts of the south, such as Charleston, S. C., and the nearby Sea Islands, where there is less white mixture and where there was malaria, some studies by Pollitzer[7] have shown much higher sickling rates in general than in northern Negroes.

HOWARD SCHNEIDER (*The Rockefeller Institute, New York, N. Y.*): May I comment on the effect of nutrition on malaria. In laboratory models it has been shown that paraminobenzoic acid deficiency, which can be achieved on a milk diet, and vitamin C deficiency result in a reduction of malaria.

For the analyses yet to come, it might be important to regard these states not as a resistance to malaria but as a diminution of susceptibility. To the extent that an organism is dependent on something that it cannot itself biosynthesize, it is dependent on the nutritional environment to furnish it. If that item should be withheld, the host animal would be much less a representative of its species in terms of the full panoply of traits that it can exhibit under normal nutritional circumstances. If we define susceptibility as the capacity to react to the pathogen with the signs and symptoms that we label as disease, it follows that if we reduce some aspect of the host that is intimately involved with this interaction between pathogen and host, disease will be diminished. This would be useful, except for the cost that must be paid, which makes us wonder whether such an approach is worthwhile. At the same time we are diminishing the host animal (in order also to diminish its capacities to respond and, hence, its susceptibility); we are making it much less of a representative of its species. This should give us pause.

Although it is true, for instance, that cows do not acquire measles, insusceptibility to measles might mean being more bovine. This choice would hardly be made by any of us.

We should prefer, in general, to be more human, whatever the whole list of attributes of being human may entail. Susceptibility to measles is certainly one.

HUTT: I get the impression that Schweitzer is a perfectionist, and I admire perfectionism. I feel that Schweitzer seeks the ultimate, 100-per cent answer, and this is commendable.

I am unhappy to detect a note of deprecation of the twin-study method. I wonder if this method is as unsatisfactory as he seems to think.

My own opinion is that in the attempts to distinguish between genetic and environmental influences, particularly with human heredity, the twin-study method has proved the very best tool for attack. I was surprised at Schweitzer's criticism of the study of Kallmann and Reisner[6] on tuberculosis. It seemed to me that this study cleared up that question better than any of the previous studies made over the last five decades.

One could say the same thing of Kallmann's studies of schizophrenia, comparing them with attempts of psychiatrists over the past 50 years to determine whether there is a genetic predisposition to it.

My question to Schweitzer is whether he thinks the twin-study method is an ideal tool for such studies.

SCHWEITZER: Osborne can answer that question better than I.

OSBORNE: First may I remind you that it was not I who deprecated the twin-study method. I am rather a strong advocate of it. I think it is an extremely useful technique, although certainly there are a number of problems in interpreting twin material that were not fully recognized in some of the earlier studies. We still have a great deal to learn before we can cope with some of these shortcomings. I do not know of a really perfect method that will answer all questions. The twin-study method certainly provides one of the most useful tools.

One thing that disturbed me about the identical-twin technique was that, in view of the theoretical stipulation that here were two individuals that were genetically identical, I naively anticipated that one would find complete concordance. Of course the concordance figures are always less than this happy event, but they are occasionally very impressive. I think Kallmann and Reisner's studies were most impressive.

Levene pinpointed this difficulty when he pointed out that the identical-twin technique replicates what essentially is an outbred individual, so that whatever its heterozygosities may be, they too are replicated.

If it should turn out, as a growing suspicion indicates, that with heterozygosity we have problems of overdominance and of plasticity to the environment, then we must expect that in a series of 1000 identical-twin pairs there would be a considerable spread over a scale of plasticity. Some pairs would be relatively unplastic and would continue concordantly to exhibit the trait in question irrevocably; in other pairs, the issue would be plastic and in doubt, but if they were not in the appropriate uniform environments the trend would be to discordance.

I agree with Schweitzer in raising the question about suitable reservations in interpreting identical-twin data. It is also true that we must find the genetic information in all ways possible in a human society as it is constructed today. I believe the identical-twin study is a very powerful tool.

Gowen: Our recent, as well as our earlier discussion, introduced the terms "identical" twins and inbreds as well as other homozygous or partially homozygous types that may be formed as consequences of continued inbreeding of relatives. Evidence gained from studies of attributes observed in individuals belonging to these types strongly influences our opinions on the relative contributions of heredity and environment in disease severity and intelligence levels. Continued re-examination of the meaning to be attached to these terms is worthwhile.

The term identical twins is used for pairs of individuals in humans, cattle, and a few other species, to identify separate organisms that presumably originated from a single reduced egg fertilized by a single sperm. The exact manner in which these twins originate is unknown in most instances. However, because of their supposed origin as individuals (tracing to the same egg and born at the same time), circular reasoning credits them with "identical" inheritance. The diagnosis is further substantiated by choosing twins, to be called identical, with closely similar features. Identical twins will individually be heterozygous in much of their inheritance. In theory they should be identical, gene for gene, if the diagnosis of "identical" is correct.

There are other ways of breeding individuals who will be closely alike in that many of their genes will be identical. Inbreeding through a single line of descent offers a means to accomplish this end. As generations of inbreeding increase, the gene pairs tend to become homozygous within and between individuals. Within the lines of descent the individuals tend to approach this state of "identity," provided that the heavy selection of heterozygous types is prevented. The similarities of the progenies of different generations, attained after some generations of inbreeding, tend to support the view that identity of the genes in the different loci has been obtained between individuals as well as within individuals. These identical twins or triplets are accompanied by a feature that is different from that of "identical twins" accomplished by some form of creation of two or more individuals from single eggs, namely, homozygosis of their genes pairs. Progenies from crosses between two different inbred strains will likewise tend to be "identicals" and will tend to be more like human identical twins, since they will be heterozygous in many particulars.

Contrasts observed in the reactions of the members of pairs of identical twins or inbred strains have been frequently utilized to prove the effectiveness of heredity or of environment in governing mental or physical development, many of the complexities of the reacting forces generally being overlooked. However it can be demonstrated that there are "peck orders" in identical twins or members of highly inbred lines as well as other noticeable divergencies in functioning and pattern for which it is hard to account. It is stimulating and clarifying to note R. S. Woodworth's critical survey of recently published material on twins and foster children[4] as a means of understanding to what extent analyses separate the effect of heredity from environment and how difficult it is to dissect their interactions. I do not think individual opinion would be changed, but the conclusions that may be drawn from twin or inbred studies will be presented with more humility.

It is customary to consider different individuals, including ourselves, as constituted of cells having, throughout, but one genotype. It is to be doubted that any individual possesses this constitution. There is growing evidence that, throughout life, accidents in cell reproduction occur to make individuals into mosaics of different types of cells. In the 2×10^{16} cells that compose adults in man many mutations would be expected. The dominants may show in competition for cell functions. The recessives may be brought to the surface by somatic crossing over, translocation, or other means. It seems probable that, as medicine advances, more attention will be given to the changing organism throughout life rather than to its stability of genotype, even though most cells retain their original structure. In theory, at least, these changes can affect the course of senescence as well as the likenesses of "identical" twins or members of inbred strains. Again, differences between individuals by these means cloud the separation of hereditary and environmental elements in the phenotype and provide an ever increasing awareness of the importance of the interactions between them. Nevertheless, with all of the evidence for the significance of heredity and/or environment, the challenge of importance is how each agent works to cause the observed changes.

Another point that has been emphasized is the question of inbreeding versus outbreeding in producing identical individuals. Inbreeding, particularly close inbreeding when properly done, is a valuable tool in bringing about similarity among individuals of the strain and genetic differences among individuals of other strains. The clear-cut advances of medical research that have come from the utilization of inbred lines speak for themselves. However, inbreeding conducted without thought and constant vigilance can lead to disaster. One of our best inbred strains of mice carries the albino gene homozygous and thus suppresses the identification of other coat-color genes. However, over the years, mutations in genes for coat color have been found within the other coat-color genes of this strain. For instance, in increasing the number of mice of this strain for large experiments, it is necessary to combine the progeny of several families of one particular generation. The under color for the original strain is brown. However, instances have been discovered where the under color had changed to black. In this particular, as in other particulars where similar changes take place, the mice are not identical. This and other changes have been traced and shown to originate in single mutant mice. Considering the numbers of genes in mice, changes of mutant origin may not be overly uncommon. Unfortunately inbred strains cannot be taken for granted even though, in general, such differences may have but small effects.

Emphasis has been given to the fact that the formation of inbred lines is accompanied by loss of heterozygosity and presumed heterosis that may affect the results. The evidence indicates that this factor may not be overly important in interpreting the manner in which genes work in the control of disease. In any case the difficulty may be overcome by utilizing F_1 crosses of different inbred lines that will be homogeneous within the cross but at the same time heterozygous. The ultimate analysis comes in the genetic segregations of the characters under study and their linkage relations.

The formation of homozygous strains may be accomplished by means other than inbreeding, as by outcrossing in species where the genetic tools are available. The homozygosity of the lines so formed may be more complete than those formed through long-continued inbreeding. The problem has interested us for about 30 years,[5] in which comparisons frequently have been made between inbred strains and homozygous types formed from outcrossing. As might be expected, homozygous individuals show somewhat more uniformity than individuals from long inbred strains. Similar differences are observed in the crosses within the two groups. It is the patterns by which the genes move through the generations of inbreeding or outcrossing that make the differences. The results are gene-dependent on gene arrangement rather than a consequence of any particular mating system by which the degree of homozygosity is attained. These results hold true for physiological systems as well as nutritional characteristics if such a separation is in any way justified.

SCHWEITZER: I am in disagreement with Schneider. He states that the study of genetics is central. I do not think such a study is central, any more than environment or agent is central. We have a series of interacting causes that lead to abnormalities or disease; nothing is central. Man is our central focus of interest, but any factor may be prominent when examined from a particular viewpoint. Many of my friends regard me as a renegade geneticist for remarks such as the one preceding; others look upon me as a Johnny-come-lately among epidemiologists, a label I equally deserve.

Now I shall comment on Hutt's query. It seems to me that when we talk about perfectionism we do not have perfection in mind. The studies in which I have participated fall far short of the ideals that were set. However, when I say that we must study disease interaction, I mean it. It may well be that genetics is a major factor, but when identical twins are studied without investigating the circumstances with respect to exposure or differences in environment, such a considerable conclusion cannot be legitimately drawn. This has nothing to do with perfection. One is justified or not, and it is only by the study of interaction of causes that the discrimination between the several variables can be made.

May I comment on the question of twin studies.

I stand on the statement that I am unable to distinguish the role of inheritance both in poliomyelitis and in tuberculosis from the twin studies. In the case of poliomyelitis there was so little additional evidence that I am still at a loss. In the case of tuberculosis there was additional evidence from studies in which heredity was examined in relation to exposure that gave me more confidence, but the factor of environment was not studied, and we know from experience the role that environment plays as a cause of tuberculosis. I do not think that the tubercle bacillus is the cause of the disease, or that either poor environment or bad genes is the paramount cause. Very often, in the prevention of disease, we may attack a lesser cause and still achieve our goal.

One last word on the question of twin studies: twins are very desirable subjects where replication in experiments is desired, particularly where we have information about, or can identify, the environmental differences that we wish to study. This is all I can say with assurance.

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Part IV. Genetic Methods for Epidemiological Investigations

AN INTERPRETATION OF FAMILIAL AGGREGATION BASED ON MULTIPLE GENETIC AND ENVIRONMENTAL FACTORS

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Introduction

Phenotypic traits investigated in genetic studies may be classified, perhaps artificially, at two levels. At the genotypic level they may be divided into traits controlled by one or a few genetic loci, often called major gene traits, or those controlled by small effects at many loci, called polygenic traits. At the phenotypic level, traits are often classified as discontinuous or qualitative, or as continuous or quantitative. These designations often lead to confusion if they are not clearly defined, as pointed out by Osborne and De George (1959). FIGURE 1 will help classify traits into four general groups based on the distributions of their genotypes and phenotypes.

Even though Mendelian genes have been assumed to be discrete units, a number of Mendelian loci affecting a single trait will produce an apparently continuous distribution. Thus we may have a few discrete genotypes which, with the influence of the environment, produce discrete phenotypes (Group A), or a continuous distribution of phenotypes (Group C). Likewise, a continuous distribution of genotypes may produce discrete phenotypes (Group D) or a continuous distribution of phenotypes (Group B).

Through the classic studies of genetic segregation and linkage, methods have been developed for the statistical evaluation of Group A traits. In fact these traits, which segregate according to theory are often chosen for study (McClintock, 1955). The methods used to analyze these simple traits in designed breeding experiments in plants and animals have been adapted to the analysis of pedigrees in man by numerous workers, including Fisher, Haldane, Finney, and Smith, as reviewed by Neel and Schull (1954) and recently by Morton (1955, 1959).

Evolutionists and animal and plant breeders have long recognized the fact that a major part of the variation within species is continuous, and methods of analysis of type B traits have been based on the concept of correlation between relatives pioneered by R. A. Fisher (1918) and Sewall Wright (1921). These results have been extended to predict and interpret the results of selection experiments as reviewed by Lerner (1959), and also generalized to take account of nonadditive genetic effects (Kempthorne, 1957).

Continuous traits influenced by discrete genic differences are sometimes found (type C). Often the distribution will have several modes, and parts of the distribution may be assigned to genotypes with some error. Urinary excretion of β-aminoisobutyric acid in man may be an example of a type C trait (Allison *et al.*, 1959). The continuity of the phenotypic distribution may often be attributed to environmental influences, poor measurement technique, and the segregation of minor genes affecting the same trait. The analysis may proceed by methods based on procedures applied to simple genetic traits as in Group A (Weber 1959, 1960).

Discontinuous traits based on a continuous genetic distribution often assume importance when animal or plant survival or susceptibility to specific common diseases in animals and man are studied. Many human family studies of common diseases are apparently concerned with type D traits.

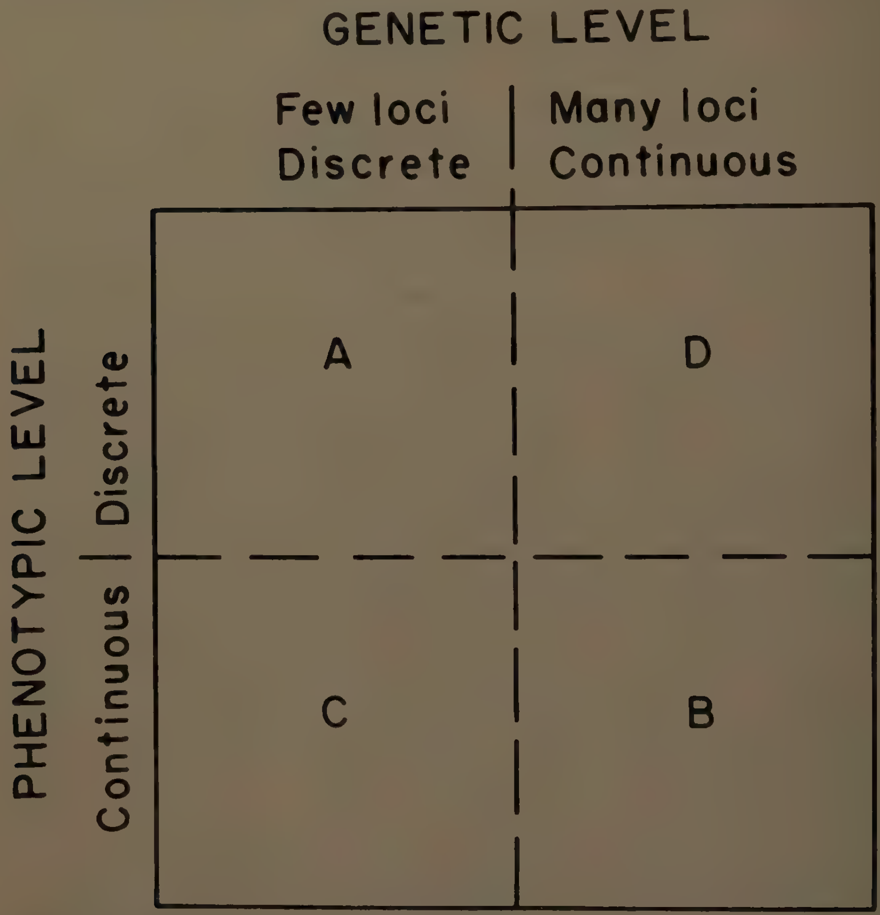

FIGURE 1. A classification of traits for genetic study based on the continuity or discontinuity of their distributions at the genetic and phenotypic levels.

Since the underlying genetic distribution is the basis on which genetic analyses rest, the decision as to what category the particular trait being studied belongs to is the first step in analysis. Often analyses may be confused by subjecting traits in the D group to classical genetic analysis, or by analyzing traits in the C group by the methods appropriate for polygenic traits. The correct decision is often difficult to make because we observe the phenotype and not the genotype, and the borderlines between continuity and discontinuity at both levels are not clear-cut.

The purpose of this paper is to present an interpretation of family studies of human disease, assuming that susceptibility to the disease is influenced by many genes and multiple environmental effects.

The Model

Wright was the first to propose a method for analysis of Group D traits. A study of polydactyly in inbred lines of guinea pigs illustrates his analysis and assumptions (Wright, 1934*a* and *b*).* Wright assumed that the ability to produce three or four toes was based on an underlying normal distribution made up of the sum of genetic and environmental components. The dichotomy was supposed to depend on a distinct threshold on the underlying scale† (FIGURE 2). Since he was able to classify parents and offspring by the number of toes they possessed, he was able to calculate a correlation between parents and offspring assuming an underlying bivariate-normal distribution function. This was done, using Pearson's (1924) tetrachoric functions. This correlation between relatives could then be interpreted by methods developed for con-

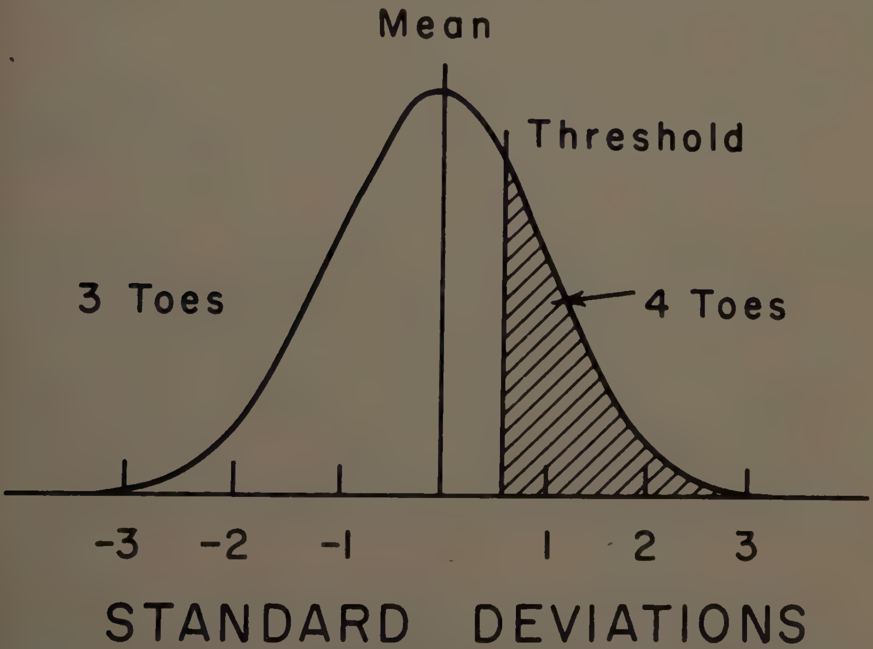

FIGURE 2. Wright's interpretation of polydactyly in guinea pigs, showing the underlying continuous normal distribution and a threshold above which four toes are observed.

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* The fact that Wright first applied this model to data obtained from inbred animals does not detract from its value for the interpretation of data obtained from random mating populations.

† This concept is essentially the rationale of the probit transformation (Bliss, 1935).

tinuous polygenic traits. This method has been extended and used in the analysis and interpretation of survival in chickens by Lush *et al.* (1948), Robertson and Lerner (1949), and Dempster and Lerner (1950). These authors also interpreted their results in terms of selection for increased survival. It should be noted that in these studies the whole population was available for study, and that because of the experimental designs environmental correlations between relatives were minimized.

In contrast, specific diseases studied in humans are of such low frequency that a whole population of families is seldom studied, and it cannot be assumed that there are no environmental correlations between relatives. The usual study begins by selecting probands having the disease in question from a population the members of which have an average probability of contracting the disease (v_x). Selection of probands from a population with a family structure may introduce bias into the study as pointed out by Haenszel (1959). Various classes of relatives are then studied to find the proportion that has the same disease (v_1). A control proportion (v_y) is determined for each class of relatives either from population statistics or by selecting control probands without the disease and investigating their relatives. If v_1 is found to be greater than v_y, a familial (not necessarily genetic) tendency is suspected.

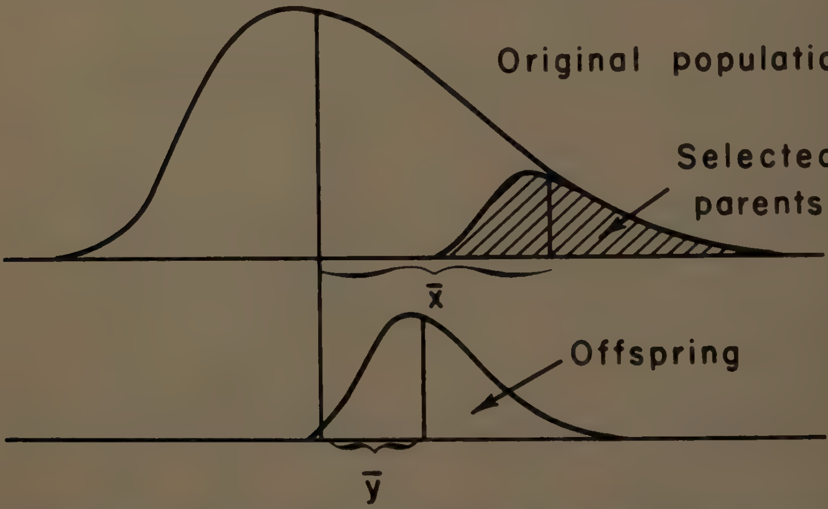

FIGURE 3. An interpretation of selection for continuous polygenic traits showing the selection differential ($\bar{x}$) and the observed shift in the mean of the next generation ($\bar{y}$) due to the selection of the parents.

If we think of this in terms of Wright's model, we find that by selecting probands we obtain people only on one side of the threshold on the underlying scale. Their position relative to the threshold is determined by their genotype and the environment in which they develop. If there is an association between the probands and their relatives, we should expect the mean of the distribution underlying the relatives to be shifted in comparison to the rest of the population. This association may be due to the environment and/or genes that the probands and relatives share in common.

This situation is comparable to the problem of selection for continuous polygenic traits in animals. FIGURE 3 illustrates the principles involved in selection experiments. All the animals in a particular generation are measured, and a group is selected to reproduce the next generation. The difference between the mean of the selected group and the original population is the selection differential ($\bar{x}$). If there is some degree of genetic control of the trait, the mean of the offspring will lie above the mean of the original population by the amount $\bar{y}$. This may be written as the regression equation:

$$\bar{y} = b\,\bar{x} + \text{error} \tag{1}$$

where b, the regression coefficient, may be thought of as the efficiency of selection, and estimated by the proportion of the selection differential that is reflected in the next generation:

$$\hat{b} = \bar{y}/\bar{x} \tag{2}$$

In animal experiments the selection efficiency is usually given a genetic interpretation because the association between parents and offspring may be assumed to be nearly free of an environmental component. Under these conditions, the selection efficiency (b) may be less than 1.0 for two reasons: (1) the phenotypic variation may be partially environmental, and (2) the selected individuals may not entirely determine the genotypes of the next generation. For example, the trait may be expressed in only one sex. Thus b may be thought of as the product of the heritability (h^2, the proportion of the total variation that is genetic) and the genetic relationship (r, the proportion of the genetic material the selected individuals have in common with the next generation):

$$b = rh^2 \tag{3}$$

Usually the genetic relationship (r) is known and the heritability (h^2) can be estimated from $\hat{b}$:

$$\hat{h}^2 = \hat{b}/r \tag{4}$$

As an illustration of the application of this concept I present the results of Falconer's (1953) experiment involving selection for high and low 6-week body weight in mice over a period of 11 generations. FIGURE 4 graphs the cumulative selection differential ($\bar{x}$) for the high line and the observed difference between the mean and the original mean for each generation ($\bar{y}$). Over the 11 generations Falconer was able to achieve a selection efficiency (b) of about 0.20.

The results of selection experiments give an estimate of what is called the observed or realized heritability. Heritabilities (h^2) are also estimated by observing correlations between relatives in unselected populations. No attempt will be made here to describe the detailed theory of correlations between relatives reviewed by Kempthorne (1957) and Lerner (1959). I assume for simplicity that we are concerned only with additive genetic variance and therefore with heritability in the narrow sense as defined by Lush (1945).*

Now we are in position to provide an interpretation of family studies in man by combining Wright's model for discontinuous polygenic traits with the concept of selection for continuous polygenic traits. The probands are analogous to the selected parents, and the relatives of the probands are comparable to the offspring.

FIGURE 5 illustrates the combined interpretation. Three normal underlying distributions are shown together with a threshold above which the individual exhibits the trait under study. The top distribution represents the population from which the probands were chosen and the proportion (v_x) of that group affected. The next distribution represents the whole population comparable

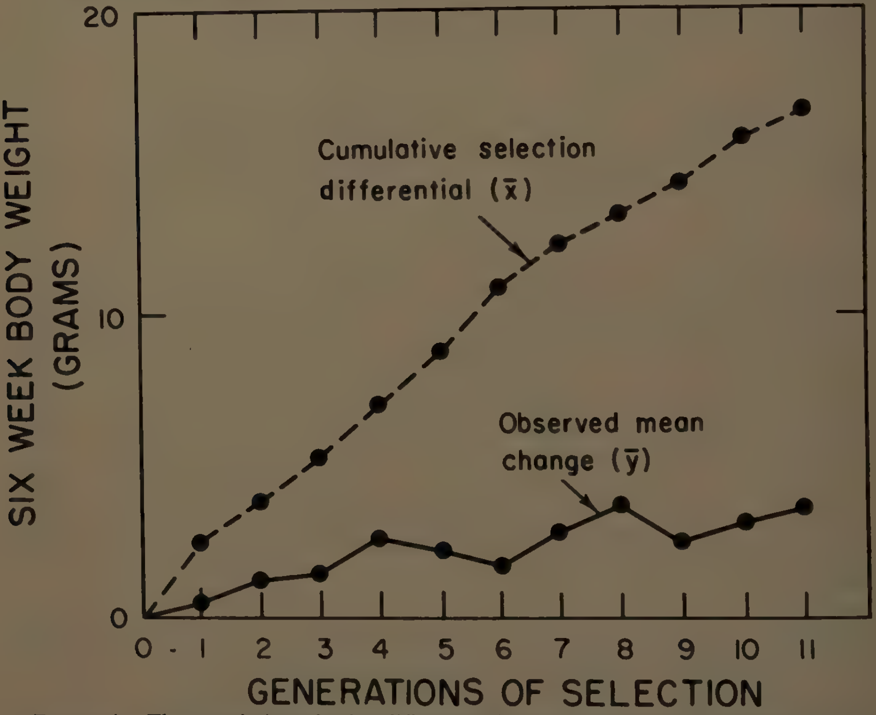

FIGURE 4. The cumulative selection differential ($\bar{x}$) and the change in the mean ($\bar{y}$) observed in 11 generations of selection for high 6-week body weight in mice. A selection efficiency ($b = \bar{y}/\bar{x}$) of about 0.20 was observed (Falconer, 1953).

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* Heritability in the narrow sense (h^2) is defined specifically as:

$$h^2 = \frac{\sigma_A 2}{\sigma_A 2 + \sigma_E 2},$$

where: $\sigma_A{}^2$ = the variance due to the average or additive effects of genes.
$\sigma_E{}^2$ = the variance due to the interaction or nonadditive effects of genes plus the environmental variance.

to the relatives of the affected probands in which a control proportion (v_y) was observed. The bottom distribution represents the relatives of the affected probands and the proportion (v_1) affected.* Estimates of $\bar{x}$ and $\bar{y}$ may be

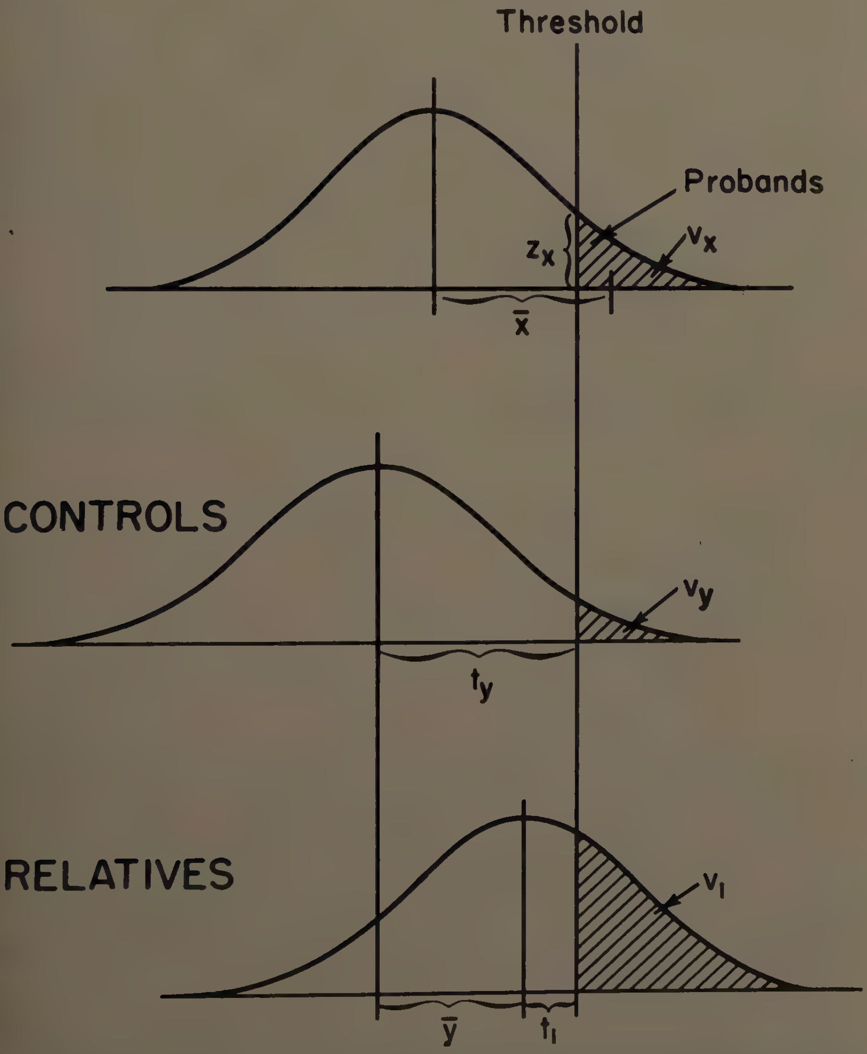

FIGURE 5. A model for the interpretation of family studies of discontinuous polygenic traits showing the normal distributions underlying the population from which probands were chosen, the control population of all people studied who are comparable to the relatives of affected probands, and the population of relatives of affected probands. The interpretation of this figure leads to Formula 5.

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* The assumption of normality of the underlying distribution is arbitrary. Even if it were normal, the distribution underlying relatives would be normal only when $b = 0.0$. However, in most studies where relatives of probands are no closer than full sibs, the true value of b would be expected to be less than 0.5 and the estimates of b would not be greatly biased.

obtained from the observed proportions using tables of the areas and ordinates of the standard normal curve, such as those found in the *Biometrika Tables for Statisticians*, Vol. 1 (Pearson and Hartley, 1954). Under these assumptions the selection differential in standard deviation units ($\bar{x}$) is given by the ratio of the ordinate of the normal curve at the threshold (z_x) to the proportion v_x in the top distribution (Pearson, 1931). The observed shift in the distribution of relatives due to the selection of affected probands ($\bar{y}$) may be estimated in standard deviation units as the difference between t_y and t_1, which are the tabled standard deviates corresponding to v_y and v_1. Thus the efficiency of selection (b) is estimated by

$$\hat{b} = \frac{t_y - t_1}{z_x/v_x} = \frac{\bar{y}}{\bar{x}} \tag{5}$$

or as before, the ratio of the observed change ($\bar{y}$) to the selection differential ($\bar{x}$).

TABLE 1

GENETIC RELATIONSHIP BETWEEN PROBANDS AND DIFFERENT CLASSES OF RELATIVES (r)

Relative	Genetic relationship (r)
Identical twin	1
Full sib	1/2
Parent or offspring	1/2
Parent or offspring (k^{th} degree)	$(1/2)^k$
Half sib	1/4
Uncle or nephew	1/4
Double first cousin	1/4
First cousin	1/8

The regression coefficient ($\hat{b}$) estimated in this way is a quantitative estimate of the association between probands and their relatives, based on the assumption of a normal distribution underlying the all-or-none expression of the trait. This association usually cannot be assumed to be entirely genetic in origin in studies of human disease. However, a genetic interpretation can be applied to give an estimate of "apparent heritability," which may be assumed to be biased upward. This estimate is obtained for each class of relatives by applying Formula **4**. The genetic relationships (r) between probands and commonly studied classes of relatives are given in TABLE 1.

Discussion of the Model

The model presented provides a framework for the interpretation of family studies of disease based on the assumptions usually made in the study of polygenic traits. Most of the theoretical development of methods for analysis of probands and their relatives has been based on the analysis of single-gene traits and linkage between them (Morton, 1959). The genetic interpretation of polygenic traits in humans has generally been confined to measurable traits (Cattell *et al.*, 1955) and to unselected groups. This has largely been carried out in twin studies as reviewed by Osborne and De George (1959).

Thus far family studies of chronic diseases have been attempts to find familial tendencies and to give a genetic interpretation on a yes-or-no basis or, perhaps, on a single-gene model. Often relative risk figures (v_1/v_y) are used to present results. Effort in these studies has been justifiably centered on obtaining unbiased comparable estimates of v_1 and v_y. This is a very important part of these studies and requires a large amount of time and care in collecting and analyzing the data as Anderson points out elsewhere in these pages.

Penrose (1953) and Edwards (1960) gave interpretations of relative risk figures for traits based on single genes and for polygenic traits not influenced by the environment. They clearly point out that the relative risk depends not only on the genetic control of a trait but also on the proportion of people with the trait in the population. The present model also shows that the relative values of v_1 and v_y depend not only on the heritability (h^2) and the genetic relationship (r) but also on the absolute values of v_x and v_y.

TABLE 2

THE ABSOLUTE (v_1) AND RELATIVE RISKS (v_1/v_y) OF DISEASE IN RELATIVES, ASSUMING FOUR DIFFERENT FREQUENCIES IN THE POPULATION ($v_x = v_y$) AND TWO VALUES FOR THE EFFICIENCY OF SELECTION (b)

Selection efficiencies	Frequency in population ($v_x = v_y$)	Absolute* frequency in relatives (v_1)	Relative risk (v_1/v_y)
$b = 0.50$	0.1000	0.3400	3.4
	0.0100	0.1610	16.1
	0.0010	0.0808	80.8
	0.0001	0.0401	400.6
$b = 0.05$	0.1000	0.1200	1.2
	0.0100	0.0140	1.4
	0.0010	0.0018	1.8
	0.0001	0.0002	2.2

* The absolute (v_1) was calculated by solving for t_1 in Formula 5. Thus:

$$t_1 = t_y - b(z_x/v_x),$$

whereas b, t_y, z_x, and v_x were known or could be obtained from the tables of the standard normal curve; v_1 then, was the area under the curve above t_1.

TABLE 2 presents absolute and relative risk figures assuming two extreme values for b (the efficiency of selection) of 0.5 and 0.05, when v_x and v_y vary over a range of rates that might be encountered in family studies. The proportions v_x and v_y are assumed to be equal in the computation of TABLE 2. In all cases as v_x and v_y become smaller, the relative risk increases. This effect is much stronger at high values of b than at low ones. Therefore, relative risk in itself is not a good quantitative measure of the association between relatives, and for valid interpretation it must be considered in conjunction with the frequency of the trait in the population.

Application

Problems arise in the practical application of this model to real family studies. In the first place the assumption that there is no environmental correlation between relatives is open to question. Also the proper choice of estimates of the rates v_x, v_y and v_1, presents problems which is discussed by Anderson elsewhere in this monograph.

For the purpose of illustrating the use of the model with published data, I correct for age differences by assuming that each person in the study has an average probability at a given age of contracting the disease before a specified older age. The choice of the interval would usually cover most of the life interval in which the disease occurs. The use of estimates of this type ignores the possibility of the genetic control of age of onset. These probabilities for the three groups, probands, controls, and relatives may be estimated to give v_x, v_y, and v_1. These estimates may be made by several different life table methods. One method was discussed by Nyholm and Helwig-Larsen (1954), which gives the probability of getting the disease before a given age assuming that no deaths occur from other causes. A more conservative method was used by Anderson *et al.* (1958), which reduces the population at risk in each age group by applying death rates from other causes, thus giving lower estimates of the probabilities.

TABLE 3

SELECTION EFFICIENCIES (b) AND "APPARENT HERITABILITIES" (h^2) ESTIMATED FROM THREE PUBLISHED FAMILY STUDIES OF BREAST CANCER

Relatives	Anderson *et al.* (1958)				Busk (1948)		Macklin (1959)	
	b*	h^2†	b*	h^2†	b*	h^2†	b*	h^2†
Sisters	0.132	0.26	0.154	0.31	0.297	0.59	0.215	0.43
Mothers	—	—	—	—	0.317	0.63	0.223	0.44
Maternal aunts	—	—	—	—	0.133	0.53	0.119	0.48‡
Paternal aunts	—	—	—	—	0.139	0.56		
Maternal grandmothers	—	—	—	—	0.000	0.00	0.123	0.49‡
Paternal grandmothers	—	—	—	—	0.000	0.00		
Cousins	—	—	—	—	—	—	0.016	0.13

* Estimated using Formula 5.
† Estimated using Formula 4.
‡ Maternal and paternal relatives were not separated in Macklin's (1959) study.

Three published family studies of breast cancer were found that either gave estimates of proportions calculated in one or both of these ways, or provided data from which they could be calculated. Since no attempt was made in any of these studies to estimate v_x, it was assumed to be equal to the v_y estimate used as the control proportion for sisters of the probands.

TABLE 3 gives the estimates of the regression coefficient (b) and the "apparent heritability" obtained. The breast cancer morbidity study of Anderson *et al.* (1958) gave 2 estimates considering the sisters of probands. The estimate in the first column used the probability that a woman of age 20 would get breast cancer before age 85, reducing the population at risk at each age group using the death rates from other causes; the second estimate was made to age 95 assuming that no deaths occurred from other causes. The relative risk obtained by the 2 methods were about the same, and the higher heritability in the second case was obtained only because the estimates of v_x, v_y, and v_1 were higher.

The second study investigated was that of Jacobsen as presented and interpreted by Busk (1948), using the Danish Cancer Registry data for the years 1942 to 44 as a source of the control proportion (v_y). This also was a morbidity study, and the results were given as the probability of a newborn girl getting breast cancer before age 90, disregarding death from other causes. This method corresponds approximately to the calculations for the second estimate using the data of Anderson *et al.* (1958). Estimates were available for several classes of relatives as shown in TABLE 3. Although higher than that calculated from Anderson *et al.*, the estimates seem very consistent over three classes of relatives, but the estimates of v_1 for the grandmothers were lower than the control proportion (v_y) obtained from the cancer registry, resulting in zero estimates of "apparent heritability." However, the control proportion obtained from the 1942 to 44 data may be very different from the corresponding rates during the lifetime of the grandmothers.

Macklin's (1959) study of deaths from breast cancer in relatives of breast cancer patients gave the last estimates. The control proportions used in estimating the heritabilities were calculated from the United States cancer mortality rates for 1914 and 1940. The use of v_y to estimate v_x is not appropriate in this case because Macklin used live probands in a mortality study. Therefore v_x is probably too low. The estimates of v_y and v_1 were made as the probability of a woman age 30 getting breast cancer before age 85, disregarding the possibility of dying from some other cause. The "apparent heritabilities" (h^2) estimated from this study were between those of the two other studies and were also consistent for the various classes of relatives except for cousins.

Discussion

Several observations are pertinent in connection with these results. The "apparent heritabilities" (h^2) observed range from intermediate to high as compared with the usual estimates obtained in studies of polygenic traits in animals. These figures are undoubtedly biased upward because of environmental similarities between relatives. However, the consistent results obtained from different classes of relatives might make one optimistic about this bias, but it must be kept in mind that probably the closer the genetic relationship, the more environmental factors individuals have in common. It is also interesting to note that a low relative risk of about 2 is compatible with a fairly high degree of genetic control of a common chronic disease such as breast cancer.

This model is suggested as a starting point for the interpretation of family studies of disease on the assumption of polygenic inheritance and multiple environmental effects. The many assumptions that must be made for its use, must be gradually checked and evaluated as more data accumulates. Extensions may readily be made to include the estimation of nonadditive genetic control of disease and the estimation of genetic correlations between different diseases or related diseases. The latter concept may help in the analysis of the genetic control of the age distribution of a disease by considering the disease occurring at different ages as different traits. However, these extensions are warranted only after the simple form of the model is found to be useful.

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STATISTICAL STUDIES OF PROBANDS AND THEIR RELATIVES

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In any field the success of research depends upon the ability to select the material and methods best suited for a given problem. In human genetics the use of proband studies has certain advantages as well as limitations, but with care in the experimental design it is possible to avoid some of the more obvious sources of error.

This discussion will be limited to reports based upon a systematic selection of probands and an evaluation of specified relatives. The term proband refers to an affected person who at any time was detected independently of the other members of the family (Morton, 1959). The first proband detected in a family is often designated as the index case or propositus, although the use of terms is not uniform.

As compared with the analysis of individual pedigrees, the proband method has the advantage of a systematic selection of cases (thus avoiding an over-representation of interesting families) but the disadvantage of a greater risk of genetic heterogeneity. The twin method can be considered a special type of proband study in which the selection of probands is limited to cotwins.

The discussion of proband studies should provide common ground for geneticists and epidemiologists, particularly in the concern for sampling bias, methods of data collection, and population rates. The topic should also hold interest for clinicians who encounter such reports in the literature or who may consider the wisdom of making such a study upon cases under their own care.

Reports of some excellent studies are already available, but they are widely scattered in different journals. As a result, the methods used for one medical condition may never come to the attention of workers interested in other diseases. It would seem desirable, therefore, to bring together the observations from reports selected to illustrate the principles involved. Attention will be directed to the statement of problems and procedures rather than to the specific conclusions from these studies.

Stating the Question

The statement of the problem is supposed to be the first step in the scientific method, but one is often tempted to bypass this phase of an investigation (Bean, 1955). As a result, inappropriate methods may be used and irrelevant data obtained, or too large a task may be attempted upon too small a sample. Kiser (1956) has summarized in a very helpful way his experiences with the statement of hypotheses for the Indianapolis, Ind., fertility study. In his opinion, the scope of the study was too wide for adequate treatment, the variables were not always precisely defined and thus not always measured, and the sample frequently proved too small. A reduction in scope would have permitted better data and larger samples.

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In preparing for a proband study, an investigator must consider four items: (1) available information concerning the nature of the disease; (2) fundamental genetic principles and previous genetic studies of this disease; (3) available sources of probands; and (4) his own interests and experience. With this background it will be easier to phrase the appropriate questions and plan the details of the study.

Nature of the disease. The problems of defining a disease are illustrated clearly by Kurland's (1957) discussion of "cerebral palsy," in which he identifies three types of definition. A *standard* definition is based upon the physical components of the disorder, as in the description by Phelps: ". . . a group of conditions which affect the control of the voluntary motor system and have their origin in lesions of various parts of the brain." A *practical* definition would be somewhat broader, including those clinical dysfunctions (motor, sensory, or psychologic) needing similar diagnostic and rehabilitation services.

Kurland then suggests that epidemiologic research requires a *limited* definition, based upon a series of exclusions: (1) clinical course, excluding progressive conditions such as Tay-Sachs cerebral-macular degeneration; (2) present knowledge of etiology, excluding congenital syphilis; (3) recognized symptom complexes, excluding hydrocephalus, mongolism, and encephalodysplasia; (4) age at action of etiologic factors, including only those "arising" from conception to the end of the neonatal (first-month) period; (5) age at diagnosis, including only those recognized prior to the end of infancy (two years).

One of the major features of Kurland's limited definition is the recognition of heterogeneity, a problem that will be illustrated from proband studies later in this paper. As Neel (1955) pointed out, "The more homogeneous the material, the greater the probability of a successful genetic analysis. . . . I am not suggesting that geneticists should display no interest in such traits, but rather that in approaching them there be a clear recognition of the methodological implications of their probable mixed nature." Neel then cited several sets of data to show that: "Once one has reduced heterogeneity to a certain point, the genetic approach may make it possible to complete the resolution."

A second important feature of a disease is the age at first diagnosis. The selection of probands and controls, the categories of relatives to be studied, and the methods used to investigate relatives will be quite different for such conditions as congenital heart disease, Huntington's chorea, or diabetes (the latter with onset from infancy to old age). In certain cases the age at onset may be important, but this is subject to the errors of retrospective recall. Unless there are specific reasons for setting age limits for the selection of probands, it would seem best to sample the entire age range. Sometimes, certain sources of probands, such as school records or rehabilitation centers, may involve an inherent age selection.

Data concerning the frequency of the condition (preferably age-specific incidence rates) are needed to estimate sample size. There is a dilemma here, however. With a relatively high frequency a larger number of patients will be available for study, but a larger proportion of the cases will have a "family history" of the condition on the basis of a random distribution alone. In addition, the genetic interpretation of the data depends upon the relative frequency of the trait, as illustrated by Crittenden elsewhere in these pages. The analysis of consanguinity, a powerful genetic method, is meaningful only when the frequency of any trait is less than about 1 in 250 (Reed, 1955).

The selection of probands and the study of relatives depends, of course, upon the available methods of diagnosis. The diagnostic methods should be reasonably well standardized with minimal observer error (Doll, 1959). In fact, genetic analysis of a given condition may have to wait until adequate diagnostic techniques become available. If the investigator intends to place much reliance upon interview data, a good lay knowledge of the condition and public willingness to discuss it are required. The methods available in earlier decades may be important if old hospital and death records are to be used. Anderson *et. al.* (1958) discussed this problem for breast cancer, and another illustration is provided by the observation that relatively fewer cases of psychosis are currently diagnosed as manic-depressive as compared with the earlier part of the century (Kramer *et al.*, 1955).

Finally, the investigator should become well acquainted with the possible biological mechanisms related to the condition under study. The literature concerning biochemical defects (Hsia, 1959) provides many illustrations of the variety of symptoms that may result from a single fundamental error in metabolism, and evidences of such variability should be looked for in the relatives. If direct genetic study of a condition is difficult, it may be of interest to explore some alternative pathways. The available evidence, for example, suggests that simple genetic factors are not important in most cases with a limited definition of cerebral palsy (Metrakos, unpublished data), but that there is a close association between prematurity and cerebral palsy (Lilienfeld and Pasamanick, 1955). Thus it would appear desirable (although presently difficult) to learn more about the role of genetics in gestational features such as prematurity or placental function (Davies, 1960).

Relevant genetic principles. It is distressing to see an occasional report in which the role of genetics in a given disorder is postulated or denied upon inadequate or irrelevant data. No one should attempt such an evaluation of the literature or of a set of data without a good understanding of at least some of the fundamental principles and concepts. Fortunately, a number of excellent source materials are now available

There are, for example, some brief accounts that, although written concerning genetic aspects of a specific disease, have much broader implications and interest: heart disease (Book, 1960), hydrocephalus (Myrianthopoulos and Kurland, 1961), mental retardation (Goodman and Herndon, 1959), congenital malformations (Fraser, 1959), and epilepsy (Metrakos and Metrakos, 1960), as well as general discussions by Penrose (1959) and by Childs and Sidbury (1957). More extensive treatments will be found in the books by Roberts (1959), Fuller and Thompson (1960), Neel and Schull (1954), and Stern (1960). For stimulating ideas concerning pathways of genetic and environmental interaction in animal disease, the book by Hutt (1958) may be recommended.

Three concepts may need some discussion here:

(1) Penetrance. In a pedigree with dominant inheritance there may be some individuals who can be assumed to possess the gene but who fail to show the

trait. The fraction (number with the trait/number with the gene) is the "per cent penetrance." Lack of penetrance is assumed to represent the action of environmental factors or modifying genes. The concept can also be applied to recessive traits.

(2) Sporadic case. A single affected individual in a family is called isolated, and such cases may be considered to be of two types. Chance isolated cases are of the same origin as familial cases, while sporadic cases arise from independent causes (such as mutation, diagnostic error, or phenocopy). Although the distinction cannot be made for a given case, the proportion of sporadic cases can be estimated by the analysis of segregating families with different patterns of mating (Morton, 1959).

(3) Relative fitness. This refers to the fitness of one population relative to that of another, where both terms are expressed as the number of livebirths ever born per newborn (Reed and Neel, 1959). The resulting percentage is used to estimate the reproductive handicap that the gene imposes upon affected persons.

A review of previous genetic studies of the disease under consideration will help the investigator to define his questions and to identify the pitfalls in experimental design that need particular attention. Any epidemiological investigations should be included in this review in order to be certain that the most important environmental variables will be accounted for.

Available sources of probands. The major issue here is that of representativeness. Certain types of analysis will require appropriate denominators based upon comparable information from population data. In addition one hopes that the generalizations from the study will hold true for a larger population. If, for example, a specific disease may be a dominant or recessive trait or be sporadic, a sample of probands should have the same proportion of these three types as does the general population.

The problem of "competing risks" (Mainland, 1955) has important implications for genetic studies. If persons with two symptoms in a syndrome complex are more likely to seek medical aid than those with only one symptom, then a clinic will not get a representative sample of the population of affected persons. In a disease that is generally mild, such as essential tremor, "it is only in special cases that such a reduction of working capacity or such severe physical or psychological disturbances arise that the tremor in itself causes the affected person to apply to a hospital or a physician for treatment" (Larsson and Sjögren, 1960). In the case of otosclerosis, a progressive disease, an affected person may not seek medical advice until both ears are involved (Larsson, 1960). It should not be surprising, therefore, if the secondary cases in a study have less severe manifestations of a disease than do the probands.

An even more serious problem would arise from a "family history bias." If a given institution has a reputation for genetic consultation, it is possible that an overrepresentation of familial cases may be referred there. For diseases having mild symptoms the probability of diagnosis (and enlistment as a proband) may be increased if there is a previously diagnosed case in the same family.

In addition to the problem of representativeness, there are three other factors to be considered in choosing a source of probands. (1) In order to obtain a desirable sample size it is often thought necessary to lump several diagnostic categories, combine several different populations of patients, or extend the time span of investigation. Each of these strategies may involve compromises, and the implications for analysis of the data should be considered in advance. (2) The quality of data may be influenced by the availability of diagnostic facilities, the adequacy of hospital and vital statistics records in the area, and the prior demographic data concerning the population. (3) The ease of obtaining data may mislead one into using the source closest at hand without considering its representativeness. On the other hand an efficient design must conserve time and expenses.

The major sources used for proband studies can be identified briefly:

(1) Most such studies use a hospital or clinic. The selective forces that regulate admission to the hospital and to the specific clinic or service must, of course, be explored.

(2) Several types of vital statistics records have been used. Woolf (1960) searched Utah death records for cases with prostate cancer. Honeyman (personal communication) is studying a series of breast and rectal cancer index cases identified through a cancer registry. Newcombe *et al.* (1959) are combining registry data concerning birth, morbidity, mortality, and marriage according to family units.

(3) Grunnet (1957) ascertained cases of diabetes through the National Diabetic Association, Copenhagen, Denmark. Mackay and Myrianthopoulos (1958) located twin probands with multiple sclerosis through the National Multiple Sclerosis Society, Washington, D.C. The selective nature of such "volunteer" sources is acknowledged.

(4) In population surveys all the sources in a given area must be utilized, and this combination provides the most representative series possible. A combination is less satisfactory when new sources are added to correct for unrepresentativeness discovered in the primary source (Grunnet, 1957). One cannot assume that the pooling of biased series will unbias the data.

The choice of probands almost always involves weighing the issues and a final compromise. There can be no serious objection if the investigator chooses the source most suitable for his purposes and understands and documents the major factors involved in the selection of the sample.

Personal interests and experience. It may be well to acknowledge the personal elements involved in research planning. Obviously one should study a given disease with a given method only if he is willing to tackle the problems involved therein. On the basis of training one person will prefer biochemical measurements while another is better equipped to work with vital statistics. One must also decide whether an approximate or a more detailed answer is necessary. There are two major questions, however, that are more deeply rooted in the history of genetics:

(1) Theoretical or practical. Steinberg (1960) states: "From the practical point of view, it does not seem to be of much value to be able to tell a parent, that, while the disease occurs in about 1 in 20,000 children in the general population, it occurs once in 15,000, or 10,000, or 5000 sibs of affected children, nor does such knowledge seem to contribute greatly to the physician's knowledge of the biology of the disease or to his efforts to find a cure for the disease. In

brief, from the practical viewpoint, nothing much is accomplished. From the theoretical point of view, such data establish that there is a genetic component of some differential importance in the causation of the disease—*provided environmental causes can be excluded.*"

The choice is sharpened by the fact that the questions asked by genetic laymen are often of the type described by Steinberg. The value of empiric risks for counseling (Reed, 1955, and Roberts, 1959) must be balanced against the need for a more theoretical analysis of genetic mechanisms in the etiology of a disease. Clearly the investigator must make a personal choice, and it would seem unfortunate if this choice were standardized along one or the other line.

(2) Simple or complex. A related alternative is outlined by Neel (1955). "I am of the opinion . . . that we are only now in a position to carry out many really basic studies on the physiology and population dynamics of these single genes, and that it would be premature to divert a large portion of our energies into studies of complex genetic situations before we have even begun to appreciate the lessons to be learned from intensive studies of single gene effects, lessons which by extrapolation may be of great value in approaching the more complex. . . . In the meantime, we may minimize the probability of future embarrassment—not to say actual opprobrium—by going no further than a statement as to heritability for many of the more complex traits."

Here again a value judgment is involved. It is true that genetic research as such is much more productive when directed toward simple traits. There are occasions, however (Neel acknowledged this in his paper), when a geneticist will be asked to help analyze a complex problem. Although he may not be able to identify a specific genetic component, it is certainly desirable that the possibility of genetic mechanisms be recognized and explored at least briefly. In this manner a geneticist can perform a service for his colleagues in other disciplines.

Qualities of a good question. This section can be summarized with a brief statement of the principal characteristics of a useful question: (1) it does not repeat questions already answered with reasonable certainty; (2) it shows an adequate understanding of genetic, clinical, and epidemiological concepts; (3) it shows an awareness of possible multiple causation; and (4) it is stated in terms that can be tested with the materials and tools available.

A Sample of Proband Studies

In studying the genetics of a character, the sequence of questions (Steinberg, 1959) appears to be: (1) Is the character hereditary? (2) If so, what is the genetic mechanism or mode of inheritance? (3) How frequent is the gene in the population? (4) What is its mutation rate? and (5) What are its linkage relations?

Steinberg further stated that "detailed analysis of hereditary patterns in man is limited for technical reasons almost entirely to characters determined by single gene differences." Crittenden elsewhere in these pages has pointed out the need for developing methods to analyze the role of quantitative inheritance in human disease. Meanwhile, proband studies can be divided roughly into two groups. For heterogeneous, broadly defined conditions (which may be quite important as public health problems) the investigation usually must be limited to the first question listed above. For the relatively rare and well-defined conditions already known to be hereditary the genetic methods of segregation and linkage analysis can be used to answer the questions remaining on the list. This distinction can be seen readily in the summary of a (nonrepresentative) sample of proband studies shown in TABLE 1.

Heterogeneity was recognized as a special problem in studies 1, 7, and 12. Lamy *et al.* (1957) showed that the relative contribution of genetic and nongenetic factors differed in five clinical subgroups of congenital heart disease (TABLE 2). Harris (1950) raised a question about the genetic heterogeneity of diabetes: Are variations in severity and in age at onset dependent on hereditary differences? In muscular dystrophy the dominant facioscapulohumeral, recessive limb-girdle, and sex-linked Duchenne types had been identified earlier, but Chung and Morton (1959) obtained evidence for an additional sporadic limb-girdle type by means of segregation analysis.

In Huntington's chorea Reed and Chandler (1958) looked for evidence of intrafamily similarities in age at onset, age at death, and interval between onset and death. Crowe *et al.* (1956) examined their data for intrafamily similarities in number of spots and in tumor site in multiple neurofibromatosis.

Some of the interesting features concerning proband selection (listed by number of study in TABLE 1) are:

(1) The patients were from all over France, but the 660 controls came primarily from Paris.

(2) One half of the probands was a "prevalence" sample (over an unstated time interval) of a research foundation that treats almost all of the children with acute leukemia in greater Boston, Mass. The representativeness of the sample cannot be determined from the data, although it is doubtful that the conclusions are seriously biased on this account.

(3) A combined twin-sib approach, in which the co-twins, sibs, and other relatives of twin probands were evaluated concurrently.

(4) The sisters of 544 patients were compared with the sisters of the patients' husbands. A smaller series of 77 breast-cancer patients were identified from hospital records for the years 1910 to 1925 in order to permit a study of daughters old enough to have developed breast cancer.

(5) A combined study of cancer at two sites. Age-specific proportionate death rates were used instead of a control group.

(6) Probands were identified from death certificates for Utah for the years 1942 to 1958.

(8) Cases were identified by a survey of a rural population in northern Sweden that had been studied earlier for previous publications.

(9) Case identification was facilitated by adequate diagnostic techniques (audiometry).

(11) A "point-prevalence" method of selection: "Those kindreds having at least one medically diagnosed choreic living in the Lower Peninsula of Michigan on April 1, 1940." A careful evaluation of the demographic and social characteristics of the affected persons was included.

(12) A combination of cases from 7 literature series and from a muscular dystrophy clinic.

TABLE 1
SUMMARY OF NONREPRESENTATIVE SAMPLE OF PROBAND STUDIES

No.	Condition studied	Place	No. probands	Nature of questions asked					References
				Herit-ability	Mode of inheritance	Frequency	Mutation rate	Relative fitness	
(1)	Congenital heart disease	France	1188	×					Lamy *et al.*, 1957
(2)	Acute leukemia, children	Boston, Mass.	249	×					Steinberg, 1960
(3)	Multiple sclerosis	United States	54	×					Mackay & Myrianthopolous, 1958; Myrianthopolous & Mackay, 1960
(4)	Breast cancer	Minnesota	621	×					Anderson, Goodman & Reed, 1958
(5)	Cancer stomach, large intestine	Ohio	312	×					Macklin, 1960
(6)	Cancer prostate	Utah	228	×					Woolf, 1960
(7)	Diabetes mellitus	England	1241		×				Harris, 1950
(8)	Essential tremor	Sweden	210		×	×			Larsson & Sjogren, 1960
(9)	Otosclerosis	Sweden	262		×				Larsson, 1960
(10)	Multiple neurofibromatosis	Michigan	137		×	×	×	×	Crowe *et al.*, 1956
(11)	Huntington's chorea	Michigan	203			×	×	×	Reed & Chandler, 1958; Reed & Neel, 1959
(12)	Muscular dystrophy	Wisconsin	800		×	×	×	×	Chung & Morton, 1959; Morton & Chung, 1959

These 12 studies were selected to illustrate a variety of disease conditions and of methods, and the full reports should serve as a good starting point for anyone interested in becoming further acquainted with proband analysis.

Designing the Procedure

A few additional general considerations are necessary before the detailed experimental plan can be laid out. The estimate of optimum sample size will usually depend upon: (1) the type of question asked; (2) the frequency of the condition in the population; and (3) the number of relatives required within a given degree of relationship; if a test of heritability is desired, two further factors may be required: (4) the rate of "familial excess" in relatives that is thought important; and (5) a choice of the appropriate significance level for the necessary statistical tests. As a rough approximation, most of the studies in TABLE 1 had more than 200 probands.

TABLE 2

RELATIVE IMPORTANCE OF GENETIC AND NONGENETIC FACTORS IN THE ETIOLOGY OF FIVE CLINICAL SUBGROUPS OF CONGENITAL HEART DISEASE*

Clinical subgroups	Relative importance of factor			
	Consanguinity	Congenital heart disease in sibs	Abnormalities during pregnancy	Other malformations in proband
Fallot's tetralogy	X	X	XXXX	XX
Pulmonary valvular stenosis	XXXX	XXXX	X	XX
Patent ductus arteriosus	XX	XX	XXXX	XX
I.V. septal defect	X	X	XXXX	XX
Atrial septal defect	XXX	XX	XXX	XXXX

* Data from Lamy *et al.*, 1957.

The method of sequential analysis (Woolf, 1960) permits the sample size to be determined by the data as they are collected. In the use of this method it would seem wise to complete the investigation of a specified number of experimental and control families before plotting additional ones, since the families most readily completed may have a biased incidence of the disease.

In an effort to increase the number of observations, it is sometimes necessary to pool data from several independent reports, even though the data may not be strictly comparable. Under favorable circumstances it may be more desirable for several investigators to agree upon enough basic details to obtain a common core of comparable data from different populations. The difficulties of collaborative research in cancer chemotherapy have been outlined by Greenberg (1959) and Mainland (1960), but proband studies would not be affected by the problems of treatment involved in the chemotherapy trials.

The limitations of retrospective analysis have been discussed recently by Dorn (1959), Haenszel (1959), Mantel and Haenszel (1959), and Cornfield and Haenszel (1960). It is becoming clear, on the other hand, that the prospective approach for proband studies may have equally severe limitations (Fraser, 1959; Reid, 1960). The major difficulty is that traits defined narrowly enough to reduce heterogeneity are so infrequent that prospective studies of a general population are very inefficient as a source of probands.

The "prospective" test of genetic hypotheses is quite different from a similar test of other etiological mechanisms. In other situations a population is surveyed to identify those with and those without a history of the possible etiological agent under test (such as smoking). These individuals themselves are then followed to determine how many of them develop the disease being tested (such as heart disease or lung cancer). A genetic test, however, involves identifying a disease history in one person and then determining whether another person (a relative) develops the same disease. The condition is further complicated by the fact that there are many relatives for each person.

Two types of prospective studies in genetics may be distinguished:

(1) In a population survey one can identify those persons who have one or more relatives (within defined categories) reported to have a given condition. The population would be followed to determine the incidence rate of the disease in those with and in those without a "family history." A special case of this type would be a prospective study of pregnancies classified before the outcome of pregnancy is known. This strategy avoids the bias in reporting that Fraser (1959) has termed "maternal memory bias" but at the expense of having to use the data without further verification (which must be minimal in such a large-scale enterprise).

(2) As an alternative, one can identify probands in some systematic manner and then study the experience of the relatives subsequent to the time that the probands were identified. If one wishes to study the children of probands in this way, the probands must be identified from records of a generation ago. This type of approach has been used for a study of mental retardation (Reed *et al.*, 1954) and for breast cancer (Anderson *et al.*, 1958). An illustration of the "subsequent risk" for a type of prospective study of sibs will be presented later in this paper.

The more specific steps for planning a proband study can be considered under the following headings: (1) the sample of probands; (2) the control group; (3) information about relatives; and (4) analysis of the data.

The sample of probands. Assuming that the proband source has been chosen, the procedure for sampling must be considered. The question cannot be dismissed by the phrases "at random" or "unselected." Many of the proband studies based upon a hospital or clinic population appear to have used an "interval-prevalence" type of sample, which would result from interviewing as many as possible of those who show up at the clinic during the time span of the investigation. If the sampling ratio is less than 100 per cent, those who attend the clinic more than once have a higher probability of interview than those who come only once. Furthermore, the sample will include those who developed the disease years ago and have survived, while others who failed to survive would be missed.

A good general principle would be to base the sampling upon persons rather than upon admissions. A sample of persons first admitted to the clinic during a specified time span would be at least representative of the clinic population, although not necessarily representative of the general population.

The following information concerning each proband would facilitate later analysis: (1) first diagnosis: age of proband, year and place diagnosis was made; (2) first admission to clinic, namely, age and date; (3) nature of referral; and (4) presenting complaint.

The total number of potential probands selected for further study should be listed, together with the number excluded for specific reasons: (1) characteristics of the proband, such as race or residence; (2) characteristics of the disease, such as any age limitation or inadequacy of diagnosis; (3) characteristics of the relatives, such as lack of information or residence in another country; and (4) refusal to cooperate. Some exclusions always appear to be necessary, and a tally of this type will permit readers of the report to make their own judgments concerning the possible bias involved.

For certain types of analysis the nature of ascertainment is important (Morton, 1959). In the case of ascertainment through an affected individual, record should be kept of each instance where two or more probands in the same family are independently ascertained. For other purposes the number of doctors who referred a given patient (not the total number of referrals) may be required.

Haenszel (1959) has discussed the "concept of case order." In many sampling schemes a given family is ascertained through the last case of the disease that has developed. Haenszel points out that a tabulation of the case-order distribution of index cases will help to determine "whether ascertained index cases reflect a distribution of affected individuals or of families with affected individuals."

The control group. In the studies listed in TABLE 1, control data were considered necessary only for the first six, those in which the role of heredity was not yet clear. In those conditions with simple inheritance, the necessary comparisons can be made between groups of families or between groups of relatives.

The difficulties in designing an adequate control procedure have been so great that Steinberg (1960) in his study of leukemia decided to use vital statistics data rather than a control group as such. As he compared the observed and expected numbers of deaths, however, he noted two points that demonstrate that population data are seldom an adequate substitute for a set of controls: (1) relatively recent vital statistics do not represent the experience of relatives living years ago, and (2) the patients' relatives are not a true cross section of the general population.

The problems in interpreting expected numbers calculated by the use of vital statistics rates can be illustrated by the data in TABLE 3. Morbidity rates (expected A), mortality rates (expected B), and a combination (expected C) were applied to the same age distributions, with quite different results. It is apparent that a single type of calculation was not equally appropriate for the three different generations. This does not argue against the use of such rates, but it does call for caution in interpreting the results.

Age- and time-specific proportional mortality rates, as used by Macklin

(1960), seem to be the best approximation available at present for proband studies of those diseases with a high mortality (such as cancer). In this method, however, analysis is limited to the dead relatives and to the death-certificate diagnoses.

If a control group is to be used, the most important variables (aside from family history) known or suspected to contribute to the etiology of the disease must be identified (Cornfield and Haenszel, 1960). On the basis of these variables a set of control index cases may be selected, each matched with one of the affected index cases. Since it is the experience of the relatives in the two groups that is compared, however, it is difficult to carry out a second matching procedure: control relative with experimental relative. As a result the data usually are analyzed as two pools. Woolf (1960) omitted the use of a control index case by matching a "control" death record with the death record of each relative in the experimental group.

TABLE 3

OBSERVED NUMBER OF RELATIVES WITH BREAST CANCER COMPARED WITH THREE TYPES OF EXPECTED NUMBERS[*]

Relationship	Number with breast cancer			
	Obs.	Exp. A[†]	Exp. B[‡]	Exp. C[§]
Mothers				
Cancer group	9	28.4	10.9	10.9
Control group	2	23.3	9.0	7.4
Sisters				
Cancer group	48	39.5	13.7	30.2
Control group	23	34.9	12.4	23.3
Daughters	5	4.7	1.3	4.5

[*] Data from Anderson *et al.*, 1958.

[†] Calculated by applying cancer morbidity rates for Iowa (1950) to the age distribution of each group of relatives.

[‡] Calculated by applying cancer mortality rates for Minnesota (1939 to 1941).

[§] Calculated by using proportional mortality rates for the dead relatives and morbidity-minus-mortality rates for the living relatives.

Hospital or clinic records may not provide a truly comparable set of controls. Even within the same hospital the different clinics may attract different segments of the population. The problems can be minimized by the careful choice of control variables, as in MacMahon and Feinleib's (1960) analysis of the effect of lactation history upon the development of breast cancer.

Anderson *et al.* (1960) used the husband's relatives as a control group in their study of breast cancer patients. Kraus (1960) has discussed the use of family members as controls in a somewhat different problem.

The development of more adequate control procedures and more appropriate general population rates would be a significant contribution to human genetics methodology.

Information about relatives. The initial information usually is obtained by an interview with the proband. The names and ages of the relatives are listed and specific screening questions are used to inquire how many are "affected"

(Metrakos and Metrakos, 1960). The details, of course, depend upon the condition under study. A pretest of the interview forms and procedure can be recommended strongly.

A next step is to approach the relatives more directly through correspondence, interview, or medical examination. If examinations are not feasible, the relevant reported conditions should be verified through hospital or vital statistics records (the loss or destruction of old records can be a serious handicap to genetics research).

The necessity of checking negative reports is shown by the data in TABLE 4 (all cases of breast cancer that developed after the first interview or letter were, of course, omitted from the analysis). If the information had not been checked routinely, the difference in breast cancer rate between the two groups would have been erroneously great. A symposium edited by Breslow and Roberts (1955) discusses procedures useful in screening for asymptomatic disease.

Analysis of the data. The analysis of the data may be limited by the manner in which the probands were ascertained. Reed and Neel (1959) pointed out that in several studies of Huntington's chorea there had been a biased selection for large (and fertile) kindreds, since the probability of ascertaining a kindred is related to the number of affected members. This bias seriously affected the estimates of relative fitness.

TABLE 4
FIRST REPORT AS COMPARED WITH MEDICAL DIAGNOSIS OF BREAST CANCERS AMONG SISTERS*

Group of relatives	Reported by informant at first interview		Diagnosis by physician	
	Total	Verified by physician	Total	Reported by informant
Sisters of propositae	29	24 (79 %)	34	24 (71 %)
Sisters of husbands	10	8 (80 %)	23	8 (35 %)

* Data from Anderson *et al.*, 1958.

It is frequently possible to determine the presence of bias and estimate its importance by an analysis of the data (Morton and Chung, 1959). A simple illustration is provided by TABLE 5. This tabulation was made to test for a possible "family history" bias in a study of breast cancer. The "subsequent experience" among sisters refers to the person-years through which each sister lived after the proposita had been admitted to the Tumor Clinic. Since these subsequent cases could not have influenced the probability of ascertaining the proposita, they can be considered unbiased in this sense. In addition, this approach could be extended to form a type of prospective genetic study of sibs, as mentioned earlier.

In diseases with a late age of onset it is difficult to estimate the "per cent affected" among a group of relatives. The proportion of relatives who have passed through the full period of exposure to risk may be different in each group thus analyzed. The details of several different methods of age adjustment were discussed by Larsson (1960), Larsson and Sjögren (1960), Myrianthopoulos and Mackay (1960), and by Anderson *et al.* (1958).

Summary

A well-designed proband study can provide meaningful answers to certain types of questions. A review of past experience should help investigators decide when to use this approach and should help them avoid some of the more obvious sources of error.

The question to be explored should be stated clearly at the outset, with due consideration for the following factors: (1) the nature of the disease; (2) the possible role of genetic factors; (3) available sources of probands; and (4) personal interests and experience.

Several recent genetical studies were evaluated to show how the experimental design is related to the questions under investigation. Discussion was limited to reports based upon a systematic selection of probands and an evaluation of specified relatives.

The experimental plan should involve general considerations (sample size, retrospective or prospective approach, collaboration with other investigators) and more specific points: (1) the sample of probands; (2) the control group; (3) information about relatives; and (4) analysis of the data.

TABLE 5
CASES OF BREAST CANCER IN PRIOR AND SUBSEQUENT EXPERIENCE OF SISTERS OF PROPOSITAE*

Year proposita was admitted	Number of breast cancers among sisters			
	Prior experience		Subsequent experience	
	Obs.	Exp.	Obs.	Exp.
To 1940	0	2.3	7	2.7
From 1940 to 1944	9	7.3	6	3.3
From 1945 on	17	20.6	6	3.2
Total	26	30.2	19	9.2

* Data from Anderson *et al.*, 1958.

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PROBLEMS AND AREAS IN GENETIC-EPIDEMIOLOGICAL FIELD STUDIES*

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During the past few years, epidemiologists and human geneticists have begun to realize that they have mutual subject matter and methodological areas of interest. Interest in genetics seems natural to the epidemiologist since he is principally concerned with studying the distribution of disease in human population groups and of those factors that influence this distribution. From such knowledge he hopes to develop leads and hypotheses regarding the role of possible etiological factors and, together with results from clinical and laboratory studies, to develop a holistic concept of what may be termed the "biology of the disease." His increased interest in human genetics may in large part be attributed to increasing concern with the epidemiological aspects of the noninfectious or noncommunicable diseases in which genetic factors appear to play a relatively greater etiological role than in the infectious diseases. Similarly from the genetic side, recent developments concerning genetic polymorphisms in man and observations on the relationship of various genetic traits to human disease have indicated to the geneticist the need for epidemiological knowledge of human disease. Most important of all, epidemiologists and geneticists have begun to appreciate the underlying operational similarities of their disciplines in that they both depend upon observational data and the derivation of inferences from naturally occurring phenomena in human population groups. Actually each discipline may be considered a subgroup of a broader discipline that might be termed human population biology. In "Genetic Methods for Epidemiological Investigations," Part IV of this monograph, several aspects of this mutuality are considered: first subject-matter areas and then methodological problems. In this paper emphasis is placed on how epidemiological studies could contribute to genetic knowledge, and the converse. This presentation is far from complete; all that can be done is to consider briefly examples of some specific areas in the hope that these will illustrate a general pattern and serve as a stimulus to further interest in these areas.

Family Studies

The first two papers of Part IV are concerned with family studies, and my first consideration is how the concept of family studies might be extended to provide additional types of data of interest to both geneticists and epidemiologists. Most family studies are concerned with the familial aggregation of specific disease entities. In some instances, however, studies have been conducted on the relationship of a biochemical characteristic and a disease. A good example of this is diabetes, for a larger proportion of relatives of individuals with diabetes have hyperglycemia than do relatives of nondiabetics. Epidemiologists have not taken full advantage of the potential usefulness of family studies as part of their interest in elucidating the relationship of biochemical or physiological characteristics with various diseases. This approach is illustrated by an example of the relationship of serum cholesterol levels to coronary artery disease, which is the subject of much current epidemiological interest.

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Several studies have suggested that coronary artery disease is aggregated in families, and there is evidence indicating an intrafamilial correlation of serum cholesterol levels.[1,2] It would therefore appear of interest to determine whether family members of individuals with coronary disease had elevated serum cholesterol levels. An exploratory study was conducted in Baltimore, Md., in cooperation with Skyring, Hammerstrom, and McKusick, in which an attempt was made to answer this question. In this study a group of males, aged 45 years and under, who had died in Baltimore during the period 1954 through 1957, was selected; heart disease had been cited as the cause of their deaths in the register of the Health Department. Clinical (particularly electrocardiographic) and autopsy records were reviewed to determine those in whom myocardial infarction was definitely diagnosed. The next death not certified as being a result of heart disease, of the same age, sex, and race was selected as a control. The surviving spouse was interviewed, and certain information concerning the deceased person was obtained. Blood was obtained from the available children as well as from the spouses, and the serum cholesterol level was determined.

TABLE 1
SERUM CHOLESTEROL LEVELS OF WIVES AND CHILDREN OF DEATHS FROM MYOCARDIAL INFARCTION AND CONTROLS

Type of family member	Family members					
	Myocardial infarction deaths			Control deaths		
	No.	Mean age (years)	Serum cholesterol: mean (±S.E.) (mg.%)	No.	Mean age (years)	Serum cholesterol: mean (±S.E.) (mg.%)
Whites						
Male children	42	13.0	231.9 (±5.31)	40	11.7	203.4 (±4.82)
Female children	34	12.9	208.9 (±6.54)	39	13.9	208.9 (±5.73)
Wives	45	39.8	273.7 (±10.34)	43	39.8	252.7 (±6.81)
Negroes						
Male children	12	9.9	218.0 (±9.42)	13	11.6	205.2 (±7.43)
Female children	13	12.8	230.2 (±11.80)	14	13.9	204.0 (±8.23)
Wives	10	42.7	282.7	8	38.1	269.3

TABLE 1 gives a summary of some of the preliminary findings; analysis of all the data has not yet been completed. For the white group the serum cholesterol levels of the wives and male children of the deaths from myocardial infarction are higher than those of the control deaths, but the only statistically significant difference ($P < .01$) is that for the male children. For the Negroes the serum cholesterol levels of the male and female children and the wives of the myocardial infarction deaths are higher than those of the controls, but none of these differences is statistically significant. These observations raise many questions. I am interested in knowing why the difference in the white group is limited to male children. Does this indicate a sex-limiting genetic factor or does it reflect a greater similarity of eating habits of males within a family? Many other questions will no doubt occur. At this point, it should be emphasized that these results must be cautiously interpreted since this study was exploratory in nature. Many problems were encountered in obtaining the data on the deaths that had occurred during a preceding time period, and some selective bias may be present; also the numbers studied were rather small. However, it was decided that these results were sufficiently provocative to warrant the conduct of a similar study on a concurrent series of deaths: a study now in progress. It would also be of interest to obtain similar types of data on siblings and perhaps on other types of relatives, but this would necessitate a large-scale undertaking.

This general approach can be extended readily to other problems in which the epidemiologist is interested, such as the relationship between various disease entities; for example, there is evidence suggesting a relationship of some forms of benign breast disease to female breast cancer.[3] It would be interesting to know whether the frequency of breast cancer among relatives of those with benign breast disease differs from that of the general population. Another area of current research activity concerns the collagen diseases, which are composed of a group of heterogenous clinical entities such as lupus erythematosus, Sjögren's disease, and rheumatoid arthritis. It would be of interest to determine whether there exists a familial aggregation not only of each of these specific diseases but of the entire group of diseases. If there is an underlying unity to these clinical entities and if genetic factors are etiologically involved as has been suggested,[4] one would expect the existence of familial aggregation of the constellation of these disease entities.

For the epidemiologist and the geneticist alike, family studies may provide one of the best means of studying genetic-environmental interactions in disease. For example, in diabetes it would be of interest to determine what environmental factors are operative in transforming a hyperglycemic individual into one with the usual clinical manifestations of diabetes. This could be studied in family members of diabetic individuals, a large proportion of whom have hyperglycemia without any clinical manifestations. The characteristics—environmental and others—of hyperglycemic family members without clinical manifestations of diabetes could be compared with those who have such manifestations. Various hypotheses that have been proposed to explain the development of clinical diabetes in susceptible individuals could be tested systematically. In so far as I am aware, families have not been studied in this manner.

These illustrations of the potential utility of family studies as a means of investigating a broad variety of genetic-epidemiological problems represent only a small fraction of those that can be conceived. I have the feeling that the epidemiologist does not appear to be as interested in family studies as formerly, when he had been concerned primarily with the communicable diseases. Perhaps the international scope of some present-day epidemiological operations has led him to ignore the family as a unit of study, but I hope that geneticists will stimulate him to return to this rather fertile area.

Environmental Influences on Genetic Mechanisms

One of the most exciting developments that has occurred in human genetics during the past few years concerns the relationship between chromosomal abnormalities and disease. For example, in the case of mongolism, it has been rather definitely established that mongoloid children have 47 chromosomes in contrast to the normal human chromosome number of 46.[5] This extra chromosome is thought to be one of the normal autosomal pairs and probably arises as a result of chromosomal nondisjunction during oogenesis or spermatogenesis. Despite the fact that this observation has changed many previous etiological concepts of mongolism, it is clear that it is not the final answer. From both the preventive and biological viewpoints, it is of extreme importance to determine what other genetic and environmental factors produce chromosomal nondisjunction in man. In this instance the genetic mechanism by means of which a specific disease develops has been determined, but one desires to go beyond the mechanism. Epidemiological studies of broad scope may provide leads or even answers as to what factors play a role.

At present the most significant clue is the observation that the frequency of mongolism increases with maternal age. Children born of mothers 45 years of age and over have more than 100 times the risk of being mongols than do those of mothers 15 to 24 years of age.[6] It thus appears that maternal age influences the frequency of nondisjunction, but one wonders in what manner maternal age exerts such an effect. Is the maternal age effect a result of a lifetime accumulation of exposures to various environmental factors, or does it reflect the constitutional changes associated with age in the maternal or paternal organism? A host of additional interesting questions can be readily raised. Looking at this matter from the viewpoint of the epidemiologist, it would be interesting to compare the characteristics of parents of mongoloid children with parents of the same ages who do not have mongoloid children with respect to a variety of factors, such as diet, occupation, ethnic background, exposure to drugs, and radiation. Various genetic traits of the parents should also be studied. Clearly, an entire series of meaningful studies could be conducted profitably in a collaborative manner by epidemiologists and geneticists.

Disease Distribution and Host Factors

Other recent developments in human genetics have focused attention on the need for additional epidemiological knowledge. For example, many human polymorphic characters such as blood groups, PTC tasting, and haptoglobins have been identified, and variations in their frequency in different population groups have been observed. Since the most likely explanation for the persistence of two or more allelomorphs in a human population in relatively high frequency seems to be that there is a selective advantage for the heterozygote, it is likely that these polymorphic characters have an influence on disease susceptibility as suggested by Ford in 1945.[7] As discussed elsewhere in this monograph, recent work has verified Ford's prediction. Leads to possible relationships of these genetic traits to disease may develop from epidemiological studies of disease frequency in various population groups as compared to the frequency of these polymorphic characters. The present situation appears to be one in which there are "genetic characteristics in search of diseases," and perhaps epidemiological studies may prove helpful in achieving this goal.

Viewing the matter from another side, epidemiological observations may suggest the need for the study of genetic or constitutional factors that may influence disease susceptibility. Some facts on lung cancer illustrate this. As is well known many studies have shown an association between cigarette smoking and lung cancer. My personal interpretation of the totality of available data is that this relationship is most reasonably explained as indicating cigarette smoking to be a major cause of lung cancer. However, it is also conceivable that susceptibility to an environmental factor such as cigarette smoking may play an etiological role since it has been estimated that a lifetime risk of developing lung cancer for a heavy cigarette smoker (who smokes more than 2 packs a day) is about 1 in 10.[8] Perhaps the 10 per cent who develop lung cancer represents a segment of the population with a particular susceptibility so that when exposed to a carcinogenic substance such as cigarette smoke, this segment has a very high probability of developing lung cancer. It would be of interest to determine those characteristics that may differentiate this possibly susceptible 10 per cent of the population from the relatively more resistant 90 per cent. Unfortunately there are only a few leads as to possible factors that should be investigated, and perhaps a "shotgun" approach should be tried. In such a study one could select a large group (about 10,000) of 2-packs-or-more-a-day male cigarette smokers, carry out a series of chemical, physical, and other determinations, and follow the group for a period of 5 to 10 years. Those who develop lung cancer could be compared with those who do not to determine in what respects they differ. Admittedly such a study would require a great deal of effort and patience, but I think it would be more than repaid by the results.

This type of follow-up study as a method of determining the relationship of disease to various host factors may probably represent the best means of obtaining answers to those questions broadly considered elsewhere in this monograph by Osborne. It may also be the most fruitful way of determining the selective advantages and disadvantages of various genetic characters. Inferences derived from such studies can be made with more confidence than those derived from cross-sectional studies of the association of disease with genetic characters.

In the papers included in this monograph a few areas have been reviewed in which it appears that geneticists and epidemiologists could collaborate with profit. Investigators have merely scratched the surface; it is certain that many types of studies of mutual interest can be conceived without any difficulty. The remainder of this presentation is devoted to a review of some methodological and inferential problems that are met in field studies, either of a genetic or epidemiological nature.

Methodological and Inferential Problems

A subject repeatedly discussed in papers on methodology of both genetic and epidemiologic studies concerns the problems associated with sampling of human population groups. It is not necessary to indicate that proper population samples are required to make adequate inferences from the sample studied. Despite this clear need for adequate sampling it is quite surprising how often data are collected on highly selected and biased groups of the population and that inferences are derived from them as if they were probability samples.

Elsewhere in these pages Anderson discusses this problem with respect to family studies and, even at the sake of repetition, I should like to consider this aspect of methodology, using as an example the results of a twin study. The material I shall present is a summary of a reanalysis of some data I obtained with Cornfield.*

Some time ago, Ronald Fisher reported the preliminary results of a study of smoking habits among adult male monozygotic and dizygotic twins.[9] He showed that 24 per cent of the monozygotic pairs were distinctly different with respect to smoking habits as contrasted to 51 per cent of the dizygotic pairs. From these data, Fisher stated, "There can therefore be little doubt that the genotype exercises a considerable influence on smoking and on the particular habit of smoking adopted, and that a study of twins on a comparatively small scale is competent to demonstrate the rather considerable differences which must exist between the different groups who classify themselves as nonsmokers, or the different classes of smokers." Recently a more detailed report of the study of these twin pairs by Todd and Mason[10] appeared together with a presentation of the raw data, thus permitting a more detailed review. The data presented by Fisher actually consisted of a pooling of three different series of twin pairs, each series having been collected in a different city in Germany. In TABLE 2 there is presented a summary of the differences and similarities in smoking habits of these different series of twin pairs.

TABLE 2
SMOKING HABITS IN DIFFERENT SERIES OF TWINS

Series	Number of monozygotic pairs			Number of dizygotic pairs		
	Smoking habits		Total	Smoking habits		Total
	Alike	Unlike		Alike	Unlike	
Tubingen	7	8	15	1	3	4
Berlin	13	8	21	6	5	11
Tubingen and Berlin	20	16	36	7	8	15
Frankfurt	13	3	16	4	13	17
Total	33	19	52	11	21	32

TABLE 2 shows that the concordance between monozygotic pairs is greater than for dizygotes in Frankfurt am Main, Germany, but not in Tubingen or Berlin. It appears that if genotype influences smoking habits, it does so in Frankfurt but not in Tubingen and Berlin. Todd and Mason[10] explain this discrepancy by pointing out that in Tubingen and Berlin, the age distributions of the monozygotes differ from the dizygotes whereas in Frankfurt they are similar. Because of the presumed effect of age and because "the method of recruitment adopted in collecting the Frankfurt list would be more likely to ensure collection of a totally unbiased sample of the two types of twins," the authors base their inferences on the Frankfurt list and discard the negative evidence of the Tubingen and Berlin lists.

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* Cornfield, J. & A. M. Lilienfeld, "Genotypic Influences on Smoking?" (unpublished data).

In evaluating these data it was possible to refer back to an early part of the paper by Todd and Mason that described how the various lists of twins were obtained. I learned that the Frankfurt list in 1952 consisted of 59 monozygotic and 85 dizygotic male same-sex pairs. However, it was found that at least 1 member of 13 monozygotic pairs and 41 dizygotic pairs were not living sufficiently close to Frankfurt to be conveniently studied. They were therefore excluded from the inquiry. After allowing for deaths and a few other changes, the questionnaire on smoking habits developed by R. Fisher was sent by O. von Verscheuer to 44 monozygotic and 46 dizygotic pairs. Completed answers were obtained from both members of 16 monozygotic and 17 dizygotic pairs. The relationship of the final respondents to the original list of twin pairs is summarized in TABLE 3. It is difficult to understand how Todd and Mason determined that "the method of recruitment adopted in Frankfurt would be more likely to ensure collection of a totally unbiased sample of the two types of twins." It is of interest that in Berlin all of the twins to whom letters were mailed replied and, in Tubingen, about three fourths replied (although not all the twins on these two lists were circularized).

TABLE 3

SELECTION OF FRANKFURT MALE SAME-SEX TWIN PAIRS USED IN SMOKING HABIT STUDY

Type of twin pair	No. of Frankfurt twin pairs on original list (1952)	No. of Frankfurt twin pairs to whom questionnaire on smoking habits was sent	No. of pairs from whom response was obtained
Monozygotic pairs	59	44	16
Dizygotic pairs	85	46	17

The fact that the results are based on inadequate samples, into which biases of many sorts may have entered, raises questions as to the validity of the inferences derived from these data. One just does not know whether to accept the findings of such a study since it was not based on what would be considered well-established sampling procedures. This statement should not be interpreted as indicating that there is no place in field investigative work for studies of possibly biased samples of human populations. Most epidemiological studies are of this nature, and elsewhere I have indicated that these studies have a rather firm place in the armamentarium of the field epidemiologist.[11] In general, however, one can only regard studies of possibly biased samples as exploratory in character and conducted in order to develop leads on which to base more definitive investigations, using more adequate sampling procedures. Any inferences derived from such studies must be interpreted with caution.

In this discussion of twin studies, it may be well to point out that herein lies an area in urgent need of epidemiological investigation. The underlying logic of the twin-study method is based on the fact that the greater degree of concordance of monozygotic twin pairs than that of dizygotic pairs reflects the greater genetic similarity of the monozygotic pairs. However, this logic carries with it the implied assumption that *ceteris paribus*, that is, monozygotic twin pairs, are no more similar with respect to nongenetic factors than are dizygotic pairs. For example, elsewhere in these pages Osborne mentions the greater similarities of monozygotic than of dizygotic twins with respect to serum cholesterol levels. Before deriving an inference that this is a reflection of genetic control of serum cholesterol levels, it would be important to determine whether monozygotic twins are more similar than dizygotic twins with respect to dietary or other environmental factors that may influence serum cholesterol levels. As a base line for the further exploitation of twin studies, it would be desirable to obtain information on monozygotic and dizygotic twins with respect to a host of environmental factors. The availability of such data would clear the way to a greater realization of the potentialities of twin studies.

Probably the closest area of kinship between human genetics and epidemiology lies in the similarity of study methods used. In contradistinction to those disciplines that are able to use the experimental method exclusively, epidemiology and human genetics are concerned with making observations on phenomena that occur naturally in human populations. In essence, data are collected concerning various types of statistical associations between diseases, or between disease and individual characteristics, or of diseases and of characteristics within families. One can never be completely certain that he has taken into account all of the variables or covariables that may have influenced the phenomenon under study. This has been noted by David and Snyder,[12] who stated that "the cogency of data on familial incidence or on twin concordance as evidence for the significant implication of genetic factors in the etiology of a disease rests ultimately upon the exhaustiveness with which it has been possible to exclude environmental factors as responsible for the associations found." In the absence of complete knowledge concerning the phenomenon under study, one is never completely certain how exhaustively one has taken into account all of the variables, genetic or environmental. Inferences derived from such data have to be made with caution since it is only by means of experimentation that one can take into account all of the variables and it is only under rare circumstances that human experimentation is feasible.

Consequently, it is necessary to depend upon an accumulation of knowledge of various kinds, including clinical, pathological, genetic, epidemiological, and animal experiments collected by different approaches and studied from different viewpoints, each datum representing one piece of the total jigsaw puzzle. It is the totality of the data that provides a pattern from which it is possible to derive fairly valid inferences concerning the biology of a disease. It is clear from the present discussion and from the other papers presented in this monograph that geneticists and epidemiologists have arrived at a point where the problems and areas of the two disciplines are so intertwined that only by close collaboration between them can we hope to arrive at a better understanding of the biological patterns of disease in human populations.

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GENETIC METHODS FOR EPIDEMIOLOGICAL INVESTIGATIONS: A SYNTHESIS

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Each of the three papers in Part IV of this monograph may be regarded as a summary of certain aspects of problems in genetic-epidemiological studies of human diseases. It is fortunate that Crittenden, Anderson, and Lilienfeld are from three different disciplines and bring out different facets of the problem for discussion. All I can do here is to add a few random thoughts connected with the points raised by these investigators, leaving the "synthesis" to readers themselves:

Genetic study of human disease goes back to the early Mendelian days; the overwhelming majority of such studies have dealt with monofactorial inheritance such as albinism and phenylketonuria. Undoubtedly, a great number of human abnormalities are controlled by a single locus. It must be equally evident that many others are not. Human geneticists have made little progress toward studying polygenic traits because of the many inherent difficulties involved. One significant progress in this direction is the empirical study of relative risk (a relative risk may be broadly defined as the ratio of two conditional probabilities). Crittenden (elsewhere in this monograph) takes one further step. He has introduced to geneticists and epidemiologists the important concept of an underlying continuous polygenic distribution for apparently discrete traits. This concept itself is not a new one; it has been used in toxicology, studies in disease resistance, and animal selection experiments for a number of years; its introduction to the analysis and interpretation of genetic data in man is new and timely. Whether breast cancer requires a threshold for expression on the underlying scale of genotypic distribution is not the point. The main significance is the introduction of the polygenic concept and a method of dealing with such a situation.

In Michael Lerner's[1] *The Genetic Basis of Selection* (1958) are mentioned several times the inheritance of an anomaly known as "crooked toes" in chickens. This condition, as many others in animals, is determined by small differences at a large number of loci with threshold for expression. When a certain fraction of these loci is homozygous, the trait is phenotypically expressed and classifiable into discrete categories. Inbreeding increases the incidence of the trait sharply. Selection by culling the individuals exhibiting the trait is virtually ineffective. If a somewhat similar physical abnormality occurs in man, most geneticists tend to think that it is a rare Mendelian recessive. The truth is, as Lerner points out, no formal Mendelian schemes can be usefully invoked even when variable penetrance and expressivity of single genes is postulated. The suggestion of Crittenden that certain human diseases, although discrete in appearance, may also have an underlying continuous distribution with a threshold level is quite consistent with our genetic knowledge of animals that have been subjected to experimentation. It is hoped that this new concept will lead to new quantitative research, analysis, and understanding of human disease.

The study of probands and their relatives to demonstrate familial association of disease has a history that probably goes back to the 1880s. One of the modern early (1938) studies of this type is that of Read *et al.*[2] entitled "The frequency of rheumatic manifestations among the siblings, parents, uncles, aunts, and grandparents of rheumatic and control patients"; its title is almost an outline of the methodology. The relatives of 33 children with rheumatic disease (probands) are compared with the corresponding relatives of 33 children without rheumatic disease (controls). It was found that the frequency of rheumatic manifestations in the former groups of relatives is significantly higher (4 times for parents and siblings) than that in the latter groups. The authors conclude that there exists strong familial tendency. However, they have also pointed out that while this may be due to "constitutional susceptibility," common exposure cannot be ruled out as a cause, and that a separate evaluation of the role of heredity and environment is not possible in their study. I mention their paper to show that basically we are still doing the same sort of thing and facing the same type of problems today.

In his discussion of the considerations that must be taken in a proband study Anderson (elsewhere in these pages) has stressed the refinements in methodology and has brought clinicians, epidemiologists, statisticians, and geneticists closer together than ever before. A clinical entity usually consists of various conditions that happen to show some common symptoms. For specific genetic studies, however, a conventional classification is of little help and, as Anderson has pointed out, most investigators must limit their attention to well-defined conditions to achieve homogeneity of material. This is of paramount importance for a comparison of the results of different workers.

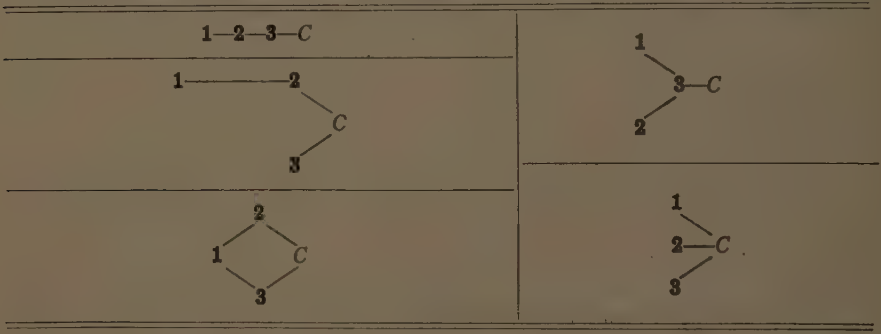
Homogeneity is, of course, also a term relative to time and knowledge. Hemophilia provides a good example to illustrate the point. A geneticist studying only family pedigrees can hardly discover the distinction between the two major types of hemophilia, since both are sex-linked recessive. One type is due to a deficiency of the plasma protein "antihemophilic factor," and another is due to a deficiency of a different protein "plasma thromboplastin component." This is a reminder that even when an apparently homogeneous condition is attained, it is no guarantee that we are dealing with a single trait. This thought has led me to the expectation that in future studies more and more attention will be directed to the primary physiological and biochemical characteristics that predispose an individual to a certain disease rather than to the end product (disease) itself. This dissecting type of study may not be considered epidemiology in the conventional sense but may nevertheless provide important clues to the understanding of epidemiological patterns observed in field studies.

When all the major sources of bias and error in a statistical study of probands and their relatives are overcome and a good size of sample is attained, what type of conclusion do we hope to be able to draw from the data? The higher incidence of a disease among relatives of probands will be more accurately determined than before, but how are we to evaluate the importance of heredity in a quantitative manner? Relative risk is apparently related to heritability. This is one of the areas that needs further clarification. The continued cooperation of workers such as Crittenden and Anderson will undoubtedly be very desirable and fruitful.

When it is assumed that a disease (or any other trait) is determined by a polygenic system, it is important to realize that this is done for the sake of mathematical simplicity. It may be as much an oversimplification as the monofactorial or difactorial hypotheses. Conceivably the etiology of a disease depends on a finite number of ultimately identifiable causes, each of which may or may not have a genetic basis, and these factors may act in a great variety of ways. Suppose that **1**, **2**, and **3** in TABLE 1 are three factors of breast cancer *C*. Some of the possible relationships are indicated in this table. It is clear that no amount of research on the resultant *C* alone can ever throw any light on the underlying pattern of causes. Lilienfeld has rightly emphasized that epidemiologists and geneticists not only should cooperate in their field studies but that their studies should also be coordinated with laboratory studies on the physiological and biochemical level. He speaks of a "constellation of diseases" in families. It follows that many factors should be studied in connection with diseases because they may share common factors.

TABLE 1

PATTERNS OF RELATIONSHIP OF CAUSAL FACTORS **1**, **2**, AND **3** TO A DISEASE CONDITION *C*



Lilienfeld (elsewhere in this monograph) and others (for example, W. J. Schull) point out that epidemiologists and geneticists have both substantive and methodological areas of mutual interest. Indeed, the study of the distribution and association of blood groups and certain diseases (such as stomach ulcer) has brought the two groups of investigators so completely together that it is impossible to say whether population genetics or epidemiology is involved. Studies of this nature will undoubtedly increase with time if a holistic concept of what may be termed the biology of the disease is to be developed.

There are times when the medical profession seems to have misunderstood genetic implications. This is evident from the numerous writings of pioneer human geneticist L. H. Snyder in the 1930s and 1940s, who tries to tell physicians that a so-called hereditary disease does not imply that it cannot be treated medically. That point has no sooner been clarified when another possible misunderstanding occurs. The present question is: Are the findings of epidemiologists and those of geneticists compatible? Can they be reconciled into one comprehensive picture? I am very happy that Lilienfeld mentioned mongolism in this connection, because it is a clear-cut example by which the question may be resolved. It is true that many of the speculated causes for mongolism may be abolished since the discovery of the trisomic condition (Lejeune *et al.*, 1959),[3] but most of the results obtained from empirical studies of mongolism still remain valid; they are not at all inconsistent with the trisomic mechanism. For instance, it is known that the incidence of mongolism increases with maternal age. In the light of the new discovery, it means simply that the frequency of nondisjunction for this particular pair of chromosomes increases with maternal (or paternal or both) age, and that is worth knowing. A mongolian trisomic behaves like a dominant lethal (not necessarily fatal) gene in a population. All or nearly all such genes arise anew each generation and are to be eliminated from the population in that very generation. The rate of nondisjunction, like the rate of mutation, may vary from population to population, from one set of environmental conditions to another, and from time to time. So the higher incidence of mongolism in one geographical area over another is, again, not inconsistent with genetic theory. More and more chromosomal aberrations in man will be found through present-day cytological technique. Such syndromes should be studied by geneticists and clinicians as well as by epidemiologists; the findings of one supplement those of others. A genetic trait also has epidemiological patterns.

In addition to the many examples given by Lilienfeld to show the interrelationship between epidemiological and genetical studies, there are instances in which the subject matters of the two fields are entirely different and yet the methodological approach is the same. To illustrate the analogy, let us consider an inbreeding problem in genetics and the smoking lung-cancer problem in epidemiology. There is no obvious similarity in these two cases, but both are dealing with certain conditional probabilities. The information on conditional probabilities, together with the initial probabilities, are sufficient to reconstruct the composition of the general population. Let R_1 be the probability of having a recessive child whose parents are first cousins, and R_0 be the probability of having a recessive (rec) child whose parents are unrelated. These are conditional probabilities that may be written

$$R_1 = P(\text{rec} \mid \text{cousins}), \qquad R_0 = P(\text{rec} \mid \text{random})$$

If c is the frequency of cousin marriages among all marriages in the population, then the composition of the entire population may be reconstructed as shown in the upper portion of TABLE 2. In most human populations the frequency c is small, probably less than 1 per cent or even less than 0.5 per cent.

Similarly, in a retrospective study we determine S_1, the probability of being a smoker if the subject has developed lung cancer, and S_0, the probability of being a smoker if he does not have lung cancer. These two conditional probabilities may be written

$$S_1 = P(\text{smoker}\,|\,\text{cancer}) \qquad S_0 = P(\text{smoker}\,|\,\text{no cancer})$$

Furthermore, if C is the frequency of lung cancer in the population from which the study is made, then the composition of the entire population may be reconstructed as shown in the lower portion of TABLE 2. Usually C is a small fraction, even smaller than c. Now we see that the method of reconstructing the general population is identical in these two cases (lung cancer may be replaced by some other disease, and the smoking habits may be replaced by some other characteristics).

TABLE 2

THE ANALOGY BETWEEN A GENETICAL PROBLEM AND AN EPIDEMIOLOGICAL PROBLEM

	Cousin parents	Random parents	Total
Recessives	cR_1	$(1 - c)R_0$	$R_0 + c(R_1 - R_0)$
Dominants	$c(1 - R_1)$	$(1 - c)(1 - R_0)$	$1 - R_0 - c(R_1 - R_0)$
Total	c	$1 - c$	1
	Lung cancer	No lung cancer	Total
Smokers	CS_1	$(1 - C)S_0$	$S_0 + C(S_1 - S_0)$
Nonsmokers	$C(1 - S_1)$	$(1 - C)(1 - S_0)$	$1 - S_0 - C(S_1 - S_0)$
Total	C	$1 - C$	1

Since the mathematical layout of these two problems is identical, a solution for one is a solution for the other. However, we are not necessarily interested in the same type of questions or comparisons that may be made in these two cases. In problems of cousin marriages the relative risk of having a recessive child, R_1/R_0, has a direct meaning, and this is what most geneticists use in practical counciling. However, we may also ask: Among all the recessives in the population, what fraction is expected to have cousin parents? From TABLE 2 we see that this fraction is ("cousins" for cousin parents)

$$u = P(\text{cousins}\,|\,\text{rec}) = \frac{cR_1}{R_0 + c(R_1 - R_0)}$$

This is the type of question raised by Lenz and Dahlberg many years ago. In retrospective study of the smoking lung-cancer problem, the relative risk of being a smoker, S_1/S_0, has only an indirect meaning. A more useful question (analogous to the one above) is: What fraction of the smokers and nonsmokers is expected to develop lung cancer? Thus from TABLE 2 we have

$$u_1 = P(\text{cancer}\,|\,\text{smoker}) = \frac{CS_1}{S_0 + C(S_1 - S_0)} \doteq \frac{CS_1}{S_0}$$

$$u_2 = P(\text{cancer}\,|\,\text{nonsmoker}) = \frac{C(1 - S_1)}{1 - S_0 - C(S_1 - S_0)} \doteq \frac{C(1 - S_1)}{1 - S_0}$$

if C is small. Hence the relative risk of developing lung cancer for smokers and nonsmokers is approximately

$$x = \frac{u_1}{u_2} \doteq \frac{S_1(1 - S_0)}{S_0(1 - S_1)}$$

This is the type of question investigated by Cornfield (1951)[4] and Woolf (1955)[5] independently. Here is another example that shows two investigators dealing with two different problems have adopted the same approach.

Suppose that the data from a retrospective study are as follows:

	lung cancer	no cancer
smokers	h	H
nonsmokers	k	K
total	n	N

Then the estimate of x is x' (then the prime may be dropped but understood)

$$x = \frac{hK}{kH}$$

The variance of x is complicated. Upon logarithmic transformation, however, it takes a very simple form. Let $y = \log_e x$. Woolf showed that

$$V(y) = V(\log_e x) = \frac{1}{h} + \frac{1}{k} + \frac{1}{H} + \frac{1}{K}$$

for large samples. When C is known and small, not only the relative risk x but the direct rates u_1 and u_2 can also be estimated; thus

$$u_1 = C\left(\frac{h}{n}\right)\left(\frac{N}{H}\right), \qquad u_2 = C\left(\frac{k}{n}\right)\left(\frac{N}{K}\right)$$

The variance of these estimates is again very simple in logarithmic scale:

$$V(y_1) = V(\log_e u_1) = \frac{1}{h} - \frac{1}{n} + \frac{1}{H} - \frac{1}{N}$$

$$V(y_2) = V(\log_e u_2) = \frac{1}{k} - \frac{1}{n} + \frac{1}{K} - \frac{1}{N}$$

An inbreeding problem in genetics, association between blood groups and disease, the risk of developing cancer from smoking and other such problems can be formulated the same way and can employ the same methodology.

Both epidemiology and genetics are well-established disciplines, but the meeting of the two is quite a new phenomenon. The merging of the two fields of study, from the historical viewpoint, is inevitable, because diseases are the end products of interaction of genetic constitution and environmental conditions. Not long ago, the mention of genetics in epidemiological circles was quite rare. In recent years the situation has been reversed; there is some indication that all unexpected variations for which the medical profession has no ready explanation are dumped into the lap of geneticists. Mutation has almost become a household word, and genetic factor has been used as an escape clause. For a professional geneticist, however, the presence of genetic factors must be demonstrated with the same rigor as a biochemical abnormality or as the presence of a pathogen is proved by laboratory tests. Little would be gained by saying merely that a certain disease is influenced by genetic factors. The meeting of the two fields is most welcome, but the cooperation should be built upon mutually enlightening research rather than on convenience.

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DISCUSSION: PART IV

SCHWEITZER: I have a question with regard to Crittenden's TABLES 1 and 3. Since the estimates are the same for relatives sharing different proportions of genes, would this be considered evidence that genetics does not play a role in this disease?

CRITTENDEN: I am not sure that I am exactly clear on that.

SCHWEITZER: Crittenden's TABLE 1 gives estimates of the number of genes that are shared in common, a figure for sibs of 50 per cent, and lower values for grandparents and cousins. The fact that the data computed from these two sets of studies are the same for different degrees for different lines would suggest that the interpretation of heredity does not apply in these cases.

CRITTENDEN: I see what the trouble is: the genetic relationship was taken into account in the heritability estimates presented in TABLE 3. The regression coefficient *b* was obtained and the heritability was estimated by multiplying by the reciprocal of the genetic relationship.

SCHWEITZER: Does this mean multiplying by 2, 4, and 8?

CRITTENDEN: That is correct. The regression coefficient *b*, obtained from relatives whose genetic relationship was one half, would be multiplied by 2 or, if the genetic relationship was one quarter, it would be multiplied by 4 to obtain the heritability estimate. TABLE 3 presents the regression coefficient *b* as well as the heritability estimates.

SCHWEITZER: I am still puzzled. What would happen if the same thing were done for measles?

CRITTENDEN: I am not sure.

LEVENE: May I raise two points about twins. First, a twin study is of use only in the stage that Anderson called stage 1; that is, where the topic of interest is whether a condition has a hereditary component. It is worthless if one desires to investigate mode of inheritance, but stage 1 is still a very big and important field.

Another question pertains to the serum cholesterol: I ask whether identical twins have diets that are more nearly alike and whether this therefore vitiates any inference of genetic control of cholesterol levels. May I point out the possibility that there is a genetic condition under which one maintains a high fat requirement as a result of which he has an appetite for fat, and much of it. This is only one method of gene action. When we say something is genetic, we do not say how the gene acts, whether through an enzyme deficiency, or a behavioral pattern, or otherwise. I think we must say, if the monozygotic twins are more alike than the dizygotic twins (this is not due to externally imposed environmental conditions, such as their being dressed alike by their parents), then this condition is genetic. We may think that only those things are genetic that are physiological; but in making this assumption I suggest that we must be very careful. Behavior is also genetic.

With respect to polymorphism, E. B. Ford said a number of years ago that if there is a polymorphic trait, one may consider the possibility of heterozygotic advantage; but in another work he defined polymorphism as the presence of two alleles in the population, both of them having greater frequency than one might expect due to mutation alone.

In the case of hemophilia of various kinds, these alleles are rather rare, and it is possible that they are maintained in the population simply by recurrent mutation.

Finally I propose to comment on the danger of significance tests. One has a tendency to conclude that there is no significant difference. Therefore nothing happens and, even worse, one group shows a significant difference; another one does not. How do we explain this? The data presented showed cholesterol levels among relatives of people who had died of a coronary disease. Among the male children there was a difference of approximately 29, with a standard error for this difference of 7, which means that the true difference might range from 15 to 43.

Among female children, the possible range of difference was from −16 to +16. The range of difference barely overlaps and there is probably a significant difference between male children and female children.

With respect to wives, the corresponding possible range is larger, due to the fact that a man has only one wife but may have more than one child; therefore there are more individuals among the children. In so far as the wives are concerned, it is unnecessary to explain why they are different from the male children because they could well be the same. In the female children there may be a difference and, of course, we know that females, if they should develop coronary disease and high cholesterol, develop it later in life.

HIRSCHHORN: I shall comment briefly on methodological difficulties discussed by Crittenden and Anderson. We have been warned by them to determine what we are studying. I wonder about Lilienfeld's study, in view of the now fairly well accepted thesis that coronary disease, especially in young males (the group selected), may have a rather close relationship to hyperlipemia or triglyceride levels with secondary variation in cholesterol. I also wonder about the validity of studying a truly secondary phenomenon without studying the primary one. The difference between male and female children might be a case in point. In a study that we made in Sweden[1] to determine the incidence of familial hyperlipemia we discovered that one of the difficulties was that, despite the use of even a fat-tolerance test (probably the most accurate way of determining whether a person is hyperlipemic), the males seemed to develop abnormality at the fasting level much earlier than the females, and it was only with the fat-tolerance test that an equality between the two sexes could be determined. This might explain the data described.

SCHWEITZER: If we know that diet is a factor in the determination of cholesterol levels, how can we impute genetics if diet is not also studied? In other words, if we want to learn the roles of genetics and diet in cholesterol levels, then we must study them in combination. Otherwise we get nowhere. I think twins are a very valuable tool for this purpose because we can investigate their diet, particularly over short periods of time, and determine how it may influence the cholesterol level. Perhaps this will illustrate what I meant in trying to limit the use of twins for certain purposes. I think this type of study is par excellence, one in which twins might be helpful. However, I demur to Levene's otherwise fine contribution and insist that until this is done we shall have no evidence of the role of genetics in this particular condition.

LEVENE: May I answer with this clarification: what I said was that if there were greater concordance in the monozygotic twins than in the dizygotic twins, this was prima facie evidence for a genetic component.

I agree with Schweitzer that while we are investigating twins we can also investigate the mechanism of this genetic difference by forcing upon twins the same diet and then forcing upon them different diets in order to determine to what extent the diet affects this mechanism; but what I maintain is that the genetic component is not something that is inborn and unchangeable. The genetic component includes everything that is controlled by genes, including a person's living habits. I grant that living habits can be changed by the physician, and this is all to the good; nevertheless we should study this. That does not make the disease nongenetic.

LILIENFELD: First I propose to comment on Levene's point. He stated that the finding of a greater similarity in behavior in monozygotic than in dizygotic twins would be a reflection of genetic factors. Thus one can visualize the following sequence of events: greater genetic concordance of monozygotic than of dizygotic twins results in greater behavioral similarities, including dietary habits that in turn produce greater similarities in serum cholesterol levels. This sequence would then be interpreted as indicating that serum cholesterol levels are genetically determined.

It seems to me that this interpretation depends on the extent to which these events are directly related to each other. For example, if genetic factors indirectly produce a greater concordance of behavior among monozygotic than among dizygotic twins simply because they are physically more alike and their interaction with the social environment is therefore more similar, the statement that genetic factors determine serum cholesterol levels is not a very profitable one. It is in the same category as a statement that everything concerning man is genetically determined since life is, in the last analysis, dependent upon genes, and many things in the environment are a result of human activity. This type of reasoning does not lead us very far in determining etiological factors in human disease; it is not a very profitable type of statement to make. In looking for genetic factors that are involved in human disease, or in physiological characteristics, we are interested in determining those specific genetic factors that are directly related to the disease or physiological characteristic.

Essentially I express agreement with Schweitzer with respect to the point he has made concerning the use of twin studies in general and, in particular, with those twin studies that have been carried out in tuberculosis and schizophrenia. Perhaps there are greater similarities in the social environment of monozygotic than of dizygotic twins, and it is this greater similarity that produces the greater concordance in disease frequency. Until we have data concerning the environmental similarities and differences of these two types of twins, it will be very difficult to use the twin-study method for obtaining definitive answers on genetic influences on disease or physiological characteristics. It seems to me that if there are two possible variables controlling the situation—genetic and environmental—it is necessary to control one in order to study the influence of the other. This is a general principle in experimentation, and all we are asking for is that this principle be adhered to in twin studies.

Concerning Levene's comment on significance tests, I agree with him in a general way. There are additional analyses that we should like to carry out on these data. For example we should like to do some regression analyses by age and other such factors. Analyses of these data have only started. I used these data, which are preliminary, merely to illustrate and to emphasize the potential value of studying the family as a unit. The family has in general been neglected by those conducting epidemiological studies of human disease. Since there is a great deal of interest in serum cholesterol and heart disease, it has occurred to me that these data might serve as an example of an additional way in which epidemiologists might look at the problem.

A comment was made that serum cholesterol levels are being replaced by triglyceride determinations with respect to heart disease. This may well be true but, again, the data that I presented are used to illustrate and emphasize the approach and method of study. Also it should be noted that when these studies were done triglyceride levels were not in vogue; perhaps, in a few years, triglycerides may be replaced by some other method of determination.

ANDERSON: The discussion points out that one of the important ways for geneticists and epidemiologists to work together is to decide when it is appropriate to carry out a study. Much time would be saved if people could get together at this ground level; moreover, if we could decide what to do and when, we could help each other most effectively.

OSBORNE: May I comment on the discussion concerning the twin-study method. The point that has been illustrated here is clearly one of the main problems of this method: it is frequently not realized what is obtained from twin data. When comparison is made of monozygotic and dizygotic twin differences, this is only a test for the presence of detectable heritability; this is the first question in any genetic problem. From this point, twin data can be fractionated to elucidate other questions, or other methods can be used for studying genetic mechanisms. Most of the frustrations with the twin study method have resulted from the fact that it was not realized, as Levene outlined, that greater concordance in monozygotic twins than in dizygotic twins is prima facie evidence for a genetic component, and that this genetic component, as measured by this method, includes everything controlled by genes, including the individual's living habits.

I now make a few final comments. The purpose of this monograph is to bring genetics more clearly into the focus of epidemiological interests by drawing from an area of experimental genetics that has particular pertinence for epidemiological problems. This experimental work involves animal populations, such as chicken populations, where there is an element of similarity to the genetic structure of human populations that is not to be found in the inbred strains of laboratory animals that are most frequently used for our models in human genetics.

We have had differing views on some important problems and, I think, through the expression of these differences the value of each view to the problems under consideration has been demonstrated. Certainly all of us have agreed on the importance of the question of genetic-environment interaction. The differences of opinions have been at what point and in what way this question should be approached.

The importance of genetic-environment interaction has relevance from quite a different aspect than from the standpoint of the biological problem involved. It relates to our objective in human genetics that has nothing to do with breeding or changing the genetic structure of our population. It relates to our aim of finding out what environmental factors, what facets of the environment, what specific environmental features need to be changed to bring into realization the full potentials of each and every one of us.

WALLACE: I make a brief reference to what has impressed me in the varying welter of opinions on certain relationships between the agricultural and the human fields.

My memory goes back to Dean Henry at the University of Wisconsin and the time when E. V. McCollum made his vitamin A and other vitamin investigations.

In those days most doctors did not know a great deal about vitamins. Agriculture happened to lead the way because Dean Henry said, as he observed the pathway of the chemist, "Let us ask the animals." McCollum agreed, and they did ask the animals. They put the question to the animals in terms that the animals could understand, and got results from them that were most fruitful.

Of course, as time goes on, people in the medical world are more knowledgeable than people in the animal world, which is altogether appropriate and right. Nevertheless, once in a while we discover something in the agricultural world that is of help.

When it comes to genetics, as Li has so forcefully pointed out, it is possible by the techniques of inbreeding, crossbreeding, backcrossing, and control of environment, through feed and uniform temperatures and other such factors, to engage in certain types of activity that enable us to measure quite precisely what is environment and what is heredity.

As we gaze at certain arrays of figures, Hutt, Gowen, and others of us who have been closer to the animal and agricultural world, marvel at the capacity of the learned gentlemen who, of necessity, are limited to inspecting data of this kind, to pour such a vast amount of philosophy into such limited data. It is a great tribute to them.

I happen to have under observation a pair of monozygotic twins. They are only 7 years old and spend some time with us every summer. I try to visualize compelling these twins to eat the same food. Curiously enough, when one is on her feed, the other is off her feed, and so it goes in a crisscross style all the time. It is a very curious psychological situation that causes them at times to desire to act opposite to each other. There is a real desire on the part of each to manifest her own individuality. A large number of these monozygotic twins must be studied in order to tell the story completely, and we must use a little psychology and observation: the kind of observation to which Li referred in such interesting fashion.

May I make one other comment that might possibly have some fruitfulness. In our inbreeding work we used a strain of chicken that has been bred by brother-sister mating for eight generations and by half brother-half sister mating for two or three more generations. The strain has been selected continually against a particular sire. We have saved those segments that combine well against that sire, with the result that we find that the particular segment of the population that combines best with that sire is the segment of the population that is less fitted to survive itself. Of 4000 hens, three weeks ago, only this particular segment, which we valued so highly because of its combining ability, contracted chronic respiratory disease (CRD). We thought that we should find it necessary to get rid of this very valuable material. You may surmise that I am making a hidden plea for doctors, and for maintaining some of the disease-susceptible population.

We administered Aureomycin and Terramycin, and this very valuable, highly inbred strain recovered rapidly. These birds will be able to continue to produce and to be of value and, as time goes on, they will very probably go through what we call the bottleneck which I envision as a kind of eventual homeostatic or new homeostatic equilibrium relative to a specific environment. Possibly in response to desperate need, something comes into being that enables these chickens that have been subjected to severe stress of long-time inbreeding finally to recover; we never know when this is going to take place, but it usually takes place in a way that ultimately is going to prove really useful.

I make a plea for the reverence of all forms of life. I hate to see any form of life disappear. I have the most profound respect for this continuing desire of doctors to understand and ameliorate the environment as it relates to individuals of various genetic composition. As a farm-paper editor, I used to answer inquiries. Questions would come in: disease, disease, disease.

In this discussion there has been talk of disease, disease, disease; so much disease that I was glad Osborne sounded a higher note. After all, it is right that those of us who are dealing with human beings, those of us who are dealing with disease (the understanding of disease, the background of disease, the degree to which it is environmental, and the degree to which it is genetic) should endeavor to understand all the ramifications that produce the varied assortment of human beings whom we love and respect: so many kinds whom, I trust, we understand. As we do that, it seems appropriate for us also to enter upon the semi-poetic level: let us ascertain that all our work ministers toward the increase of joy, beauty, and creativity in the fields of joy and beauty that mankind was meant to serve.

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